International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Human Health Risk Assessment and Effectssome Heavy Metals in the Tissue of Two Species of Cichlidae (*Hemichromisfasciatus and Tilapia zillii* × *Tilapia guineensis*) from the Western Part of the Ebrie Lagoon, Côte D'ivoire

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Abstract*: *This study has been conducted to assessment the human health risk via the consumption for Hemichromisfasciatus and zillii × Tilapia guineensis by contamination of heavy metal such as Hg, Pb, Cd and As. Fishes species were collectedin the mainland and maritime areas in western part of Ebrie lagoon. Moreover, Histopathological alterations were used as indicators for the effects of anthropogenic pollutants. After dried and digested of tissues (muscle, liver and kidney), the concentration of lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) in tissues were carried out using an atomic absorption spectrophotometer. For histopathological studies, tissues (liver and kidney)were screened for a variety of histopathological features and lesions by light microscope under 400X magnifications. The Hazard Index (HI) for all heavy metals in the species were higher than 1. The highest HI value suggests a potential health risk by fish consumption of the population.Histopathological examination of organsof fish species revealed varying alterations. Mostly, the damages of liver and kidney of both fish speciesincluded haemorrhage, fibrosis, congestion in blood sinusoid, dilatation of blood sinusoid, cytoplasmic vacuolar, necrosis, degeneration of cells, dilatation vacuolar and intravascular haemolysis in blood vessels. However, the species of Hemichromisfasciatus presented the damage in all organsobserved.

Keywords: Target hazardous quotient,Hazard index, tissue, human health risk, histopathological

1. Introduction

Lagoons are highly productive ecosystems. They contribute to the overall productivity of coastal water. They also provide essential habitat for many fish species (Anthony et al., 2009). Despite, lagoons suffer multiple pressures from uses and activities that threaten their ecological integrity (Angel, 2019). They are seriously threatened by severe pollution from different sources (Esteves et al., 2008). In Côte d'Ivoirethe Ebrie lagoon, despite its ecological value and its significant aquaculture exploitation is the receptacleof domestic, agricultural and industrial waste water. The studies revealed a direct relation between wastewater spills and the metallic pollution increasing of the Ebrie lagoon (Coulibaly, 2013; Inza et al., 2015; Daniel et al., 2016).Some metals such as lead, cadmium, mercury and arsenic as major pollutants because of their toxicity. Moreover, they are persistent and tend to bioaccumulate in the food chain posing a risk to humans and ecosystems (EPA, 2009).

Toxicity of heavy metals can affect individual growth, physiological function, fish reproduction and cause histological changes in fish tissues (Amundsen, 1997). Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on aquatic biota and can constitute a danger on health for the entire population in the ecosystem (Mohamed, 2009). Recent several studies indicated that the alterations in fish tissues can be the toxic effects of heavy metals (Coulibaly et al., 2012; Salim, 2015; Abarghoei et al., 2016; Samuel et al., 2017; Kaur et al., 2018; Ibrahim et al. 2019). The observations on histopathology include several lesions such as cytoplasmic degeneration, necrosis, hemorrhage, etc…in fish tissues exposure to heavy metals.

Few studies have conducted on the histological changes of fish tissues collected in Ebrie lagoon (Coulibaly, 2013; Yapi, 2014). In the western part of the Ebrie lagoon, near the cities of Jacqueville and Dabou, where several human activities are taking place, pollutions are more frequent. In mainland area, agricultural activities (local plantations of banana and coconut), fishing activities, industrial activities were much practiced. These activities constitute a potentialsource of heavy metals in lagoon and could beendanger for ecosystems and public health through consumption fishes contaminated.

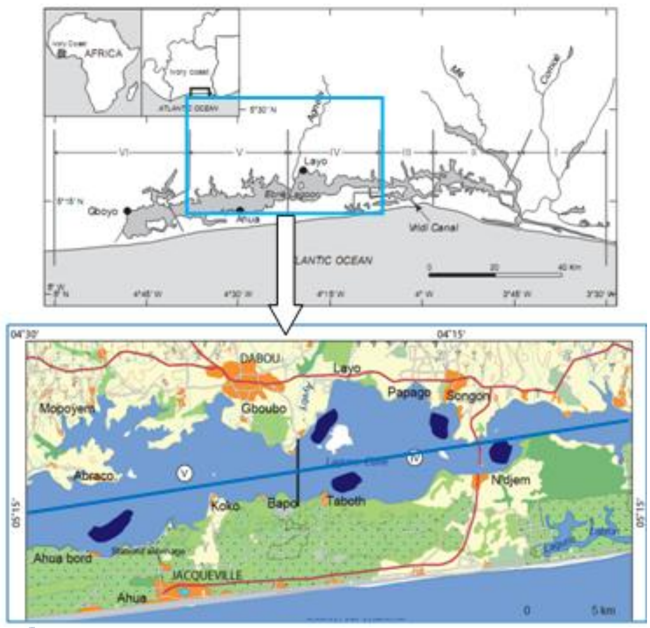
The aim of the present study was to assessment the human health risk for *Hemichromisfasciatus* and *zillii* × *Tilapia guineensis* consumption and determine histological changes liver and kidney of these fishes from the western part of Ebrie Lagoon.

2. Material and Methods

2.1 Tissues sample analysis

360 fish specimens (*Hemichromisfasciatus* and *Tilapiazillii* × *Tilapia guineensis)*were collected about each month, due

to 30 specimens per species. The Fishes samplingcarried outfrom February 2014 to January 2015 at five stations (N'djem, Taboth, Ahua, Layo and Songon) on the maritime and the mainland areas of the western part of the Ebrie lagoon (5 ° 13'15 "N and 4 ° 42'0" W) (Figure 1).The fish were caught with a gill net by professional fishermen and transferred alive in a cooled ice box (4°C) to the laboratory. Two analyses were performed. After measurement and dissection, target organs (muscle, liver and kidney) were quickly remove. To determinate heavy metals concentration,the organs were then dried and then digested using a microwave, digestion in a closed microwave oven. The determination of heavy metals was performed in accordance in with the method described by EPA (2007). The analyses of lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) were carried out using an atomic absorption spectrophotometer. For histopathological studies**,** the organs samples (liver and kidney) obtained from each species were fixed inBouin’s solution for 24 h at room temperature. The fixed tissues and dehydrated were embedded in paraffin wax and sections of 4 µm were cut by using microtome. Sections were deparaffinized in toluène, hydrated in ethanol and stained with hematoxylin – eosin (HE) method.Tissues were screened for a variety of histopathologicalfeatures and lesions by light microscope under 400X magnifications.



The blue spots blue indicates the sampling station

Figure 1: Location of sampling areas in the mainland area and maritime area (Ebrie Lagoon, Ivory Coast).Mainland area (Songon, Layo), maritime area (Ahua, Taboth,N’djem)

2.2 Data analysis

In the study, we employed the health risk assessment model generated by United States Environmental Protection Agency (USEPA, 1989) to assess the human health risk of heavy metals to adults. The target hazardous quotient (THQ) is used commonly for the assessment of the potential of non-carcinogenic risks associated with long term exposure to contaminants, such as heavy metals from food such as fish and water (USEPA, 1999):

$$THQ = \frac{(EFr\ X\ ED\ X\ Fr\ X\ C)}{(RfD\ X\ BW\ X\ AT)}$$

With THQ is the target hazard quotient; EFr represents the exposure frequency which was in this study (365 days/year). ED is the exposure duration (30 years for non-cancer risk as used byUSEPA (2011), Fr is the fish ingestion rate (0,044 kg/person/day) (MIRAH,2016); The C represents the metal concentrations (mg/kg. wet weight) in the tissues of fish samples;the oral reference dose (RfD) was adopted from the (USEPA, 2009) (Table 1);BW is the average body weight (bw) (70 kg); AT is the average exposure time for non-carcinogens (EF×ED) (365 days/year x 30 years) (USEPA, 2011) if the THQ value is < 1.00 that means the exposed population is supposed to be safe; however, when THQ > 1.00 there is a potential risk related to the studied metal in the exposed population.

Table 1: Oral reference doses of heavy metals (USEPA, 2009).

Heavy metals	Hg	Pb	Cd	As
RfD(mg/kg-day)	$1.6\ X\ 10^{-4}$	$1\ X\ 10^{-3}$	$4\ X\ 10^{-3}$	$3\ X\ 10^{-4}$

For the risk assessment of multiple metals containing in fish, a total HI was employed by summing all the calculated THQi values of metals as described in Equation (USEPA, 1989).

$$HI = \sum_{i=1}^{n} THQi$$

HI is the hazard index for the overall toxic risk and n is the total number of metals under consideration. If, HI<1.0, the non-carcinogenic adverse effect due to this exposure pathway or chemical is assumed to be negligible.

3. Results

3.1 Mean concentration of heavy metals in the tissues

The results on metals concentration in organs show that as content was the highest and that of Cd was the lowest. Hg, Pb, Cd and As concentrations showed maximum in Mainland areas. The concentration of heavy metals in the organs of fish samples followed the following order kidney> liver> muscle in both areas.There are significant differences ($P < 0.05$) in the levels of all metals among organs. The results showed that the kidney has the highest values of heavy metals in both species, while the lowest concentrations was observed in the muscles. *H. fasciatus* accumulates more heavy metals in its organs than the *T. zillii × T. guineensis* hybrid (Table 2).

Table 2: Mean concentrations of heavy metals (mg/kg dry weight) in muscle, liver and kidney of the *Hemichromisfasciatus* and Tilapia Hybrid (*Tilapia guineensis xTilapia zillii*) in Mainland and Maritime areas fromEbrie lagoon from February 2014 to January 2015

Heavy metals	Species	Areas	Muscle	liver	kidney
Hg	H.f	Mainland	0.18±0.02[a]	0.29±0.04[b]	0.38±0.08[1,c]
		Maritime	0.18±0.03[a]	0.29±0.06[b]	0.41±0.09[2,c]
	Tz x Tg	Mainland	0.11±0.04[a]	0.24±0.07[2,b]	0.28±0.06[1,c]
		Maritime	0.12±0.05[a]	0.21±0.06[1,b]	0.31±0.10[2,c]
Pb	H.f	Mainland	0.17±0.03[a]	0.27±0.06[1,a]	0.52±0.29[2,b]
		Maritime	0.19±0.06[a]	0.30±0.11[2,b]	0.39±0.10[1,b]
	Tz x Tg	Mainland	0.11±0.05[1,a]	0.19±0.10[1,b]	0.40±0.30[2,c]
		Maritime	0.15±0.07[2,a]	0.25±0.09[2,b]	0.36±0.24[1,c]
Cd	H.f	Mainland	0.08±0.05[1,a]	0.20±0.07[1,b]	0.26±0.08[1,c]
		Maritime	0.19±0.32[2,a]	0.35±0.27[2,ab]	0.49±0.37[2,b]
	Tz x Tg	Mainland	0.04±0.04[1,a]	0.12±0.05[1,b]	0.17±0.08[1,c]
		Maritime	0.08±0.08[2,a]	0.19±0.10[2,b]	0.30±0.13[2,c]
As	H.f	Mainland	0.28±0.11[a]	0.62±0.25[b]	1.49±0.65[2,c]
		Maritime	0.27±0.12[a]	0.60±0.58[a]	1.07±0.76[1,b]
	Tz x Tg	Mainland	0.19±0.08[a]	0.29±0.10[1,a]	0.93±0.66[2,b]
		Maritime	0.18±0.09[a]	0.41±0.39[2,a]	0.90±0.76[1,b]

Superscript letters in the same row show differences among organs

Superscript numbers in the column show differences among areas

3.2 Human health risk assessment:

The target hazardous quotient (THQ) and Hazard index (HI) of heavy metals by consuming *Hemichromisfasciatus* and *Tilapiazillii* × *Tilapia guineensis* collected in mainland and maritime areasfor an individual adult are presented in Table 3& 4. In this study, the THQ value of Hg, Pb, Cd, and As in fish species were < 0.1.The THQ for heavy metals decreased in the following order: Hg> As > Cd >Pb. However, the total non-carcinogenic hazard index (HI) value for allconsidered metals through multiple exposure pathways was above 1(1.33 and 1.38 for *H. fasciatus* of the both areas and1.03for *T.zillii* × *T.guineensis* in the mainland area) except*T. zillii* × *T.guineensis* collected in maritime area with HI=0. 92, which is higher than the safe level. The total THQ values (HI) of metals range at the following sequence: *Hemichromisfasciatus*>*Tilapia zillii* × *Tilapia guineensis*. The potential health risk by *Hemichromisfasciatus* species consumption was greater than Hybrid tilapia consumption.

Table 3: Estimated target hazard quotients (THQ) for individual metals and HI from consumption of *Hemichromisfasciatus* and *Tilapia zillii* × *Tilapia guineensis* collected in the mainland area.

Fish species	Hg	Pb	Cd	As	HI
Hemichromisfasciatus	0.71	0.03	0.05	0.59	1.33
Tilapia zillii × *Tilapia guineensis*	0.43	0.17	0.03	0.40	1.03

Table 4: Estimated target hazard quotients (THQ) for individual metals and total HI from consumption of *Hemichromisfasciatus* and *Tilapia zillii* × *Tilapia guineensis* collected in the maritime area.

Fish species	Hg	Pb	Cd	As	HI
Hemichromisfasciatus	0.71	0.03	0.07	0.57	1.38
Tilapia zillii × *Tilapia guineensis*	0.47	0.02	0.05	0.38	0.92

3.3 Histopathological effects

In the study, 100% of *Hemichromisfasciatus* organs observed present deteriorations against 92% of alterations in organs *Tilapia guineensis* x *Tilapia zillii*. Liver of the fish *Tilapia guineensis* x *Tilapia zillii* and fish *Hemichromisfasciatus* inhabiting the water of both areas mainland and maritime suffered from many pathological alterations. The observed histopathological alterations of liver cells in two species include focal areas of necrosis, fibrosis, haemorrhage, vacuolar degeneration and cytoplasmic vacuolar. In addition, hepatic lesions in the liver tissues of *H.fasciatus* were characterized by congestionin blood sinusoid, dilation of sinusoids, intravascular haemolysis in blood vessels and degeneration of the hepatocytes. While, haemosidrin and pyknotic nuclei in liver tissues of Tilapia species were also observed (Figure2& 3).

Histopathological alterations were detected in the kidney of *Tilapia guineensis* x *Tilapia zillii* and *Hemichromisfasciatus* collected from mainland and maritime areas. The pathological in the kidney tissues of species results included focal areas necrosis, haemorrhage, and hypertrophy cells. Also, kidney pathology of *Hemichromisfasciatus was* characterized by melanomacrophage aggregates and fibrosis. Severe degeneration and aggregation of inflammatory cells were observed in kidney tissues of *Tilapia guineensis* x *Tilapia zillii* (Figure4 & 5).

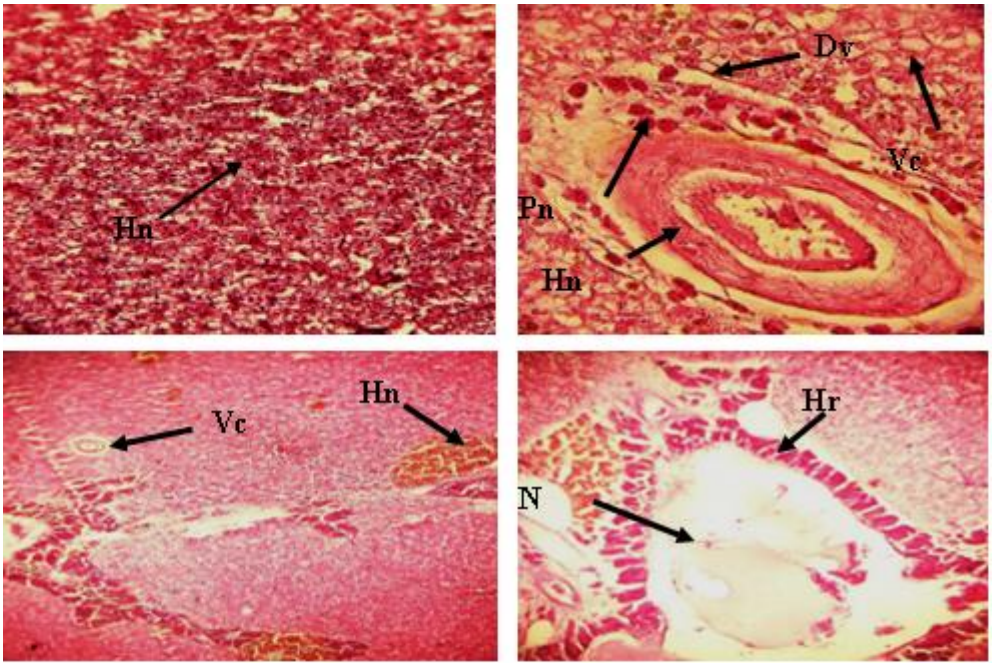


Figure 2: Photomicrograph of the liver of Tilapia guineensis x Tilapia zillii sampled from mainland and maritime areas, western part of Ebrie Lagoon (Ivory Coast):Hrhaemorrhage; F Fibrosis; Pnpyknoticnuclei;Hnhaemosidrin ;Vc cytoplasmic vacuolar; N focal area of necrosis ; Dv Dilatation vacuolar.. (Hématoxylin- Eosin. X 400).

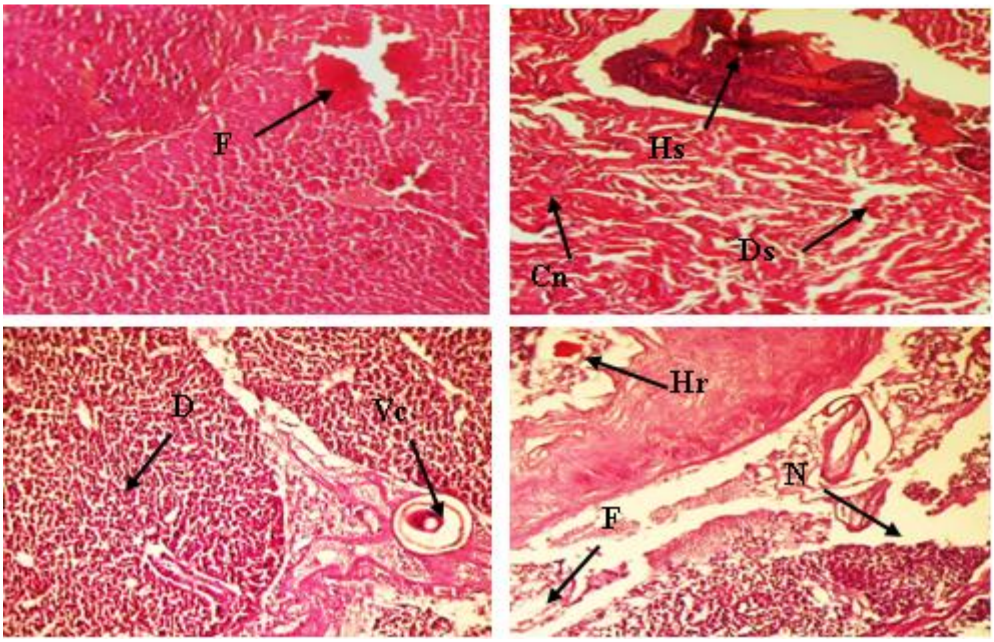


Figure 3: Photomicrograph of the liver of Hemichromisfasciatus sampled from mainland and maritime areas,western part of Ebrie Lagoon (Ivory Coast): Hrhaemorrhage; F Fibrosis; Cn Congestion in blood sinusoid; Ds Dilatation of blood sinusoid; Vc cytoplasmic vacuolar; NNecrosis;D degeneration of the hepatocytes; Dv Dilatation vacuolar; Hs intravascular haemolysis in blood vessels**.** (Hématoxylin- Eosin. X 400).

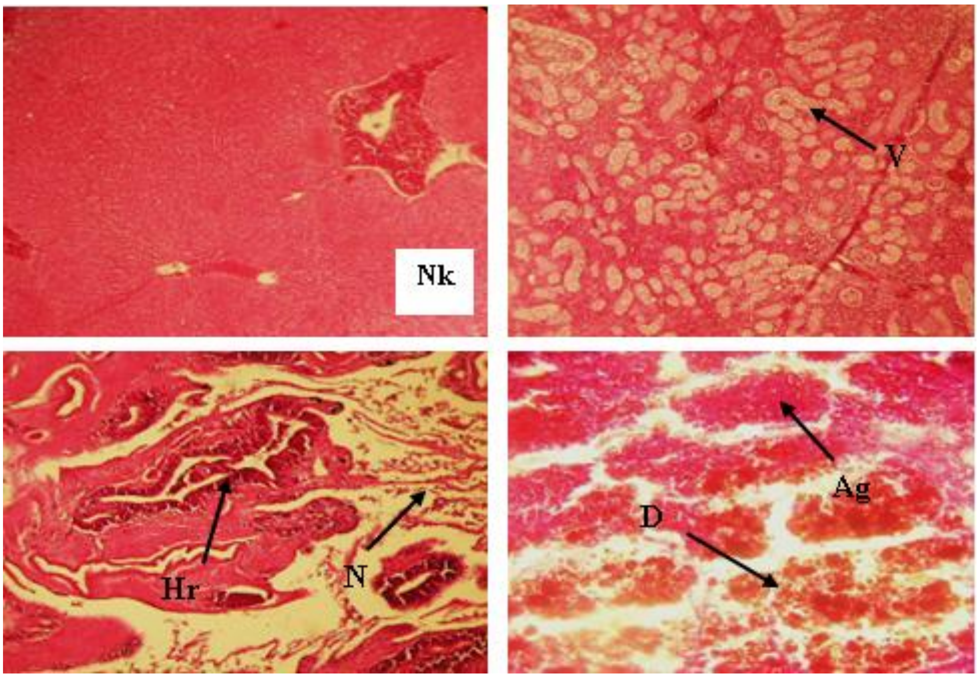


Figure 4: Photomicrograph of the kidney of Tilapia guineensis x Tilapia zillii sampledfrom mainland and maritime areas, western part of Ebrie Lagoon (Ivory Coast):Nk Normal kidney; V vacuolar of tissue; Hp Hypertrophy of cells; Ag Aggregation of inflammatory cells; N focal area of necrosis; Hrhaemorrhage. (Hématoxylin- Eosin. X 400).

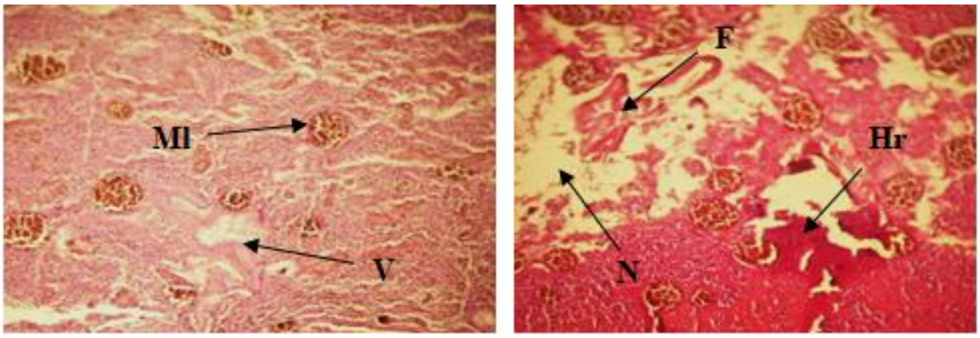


Figure 5: Photomicrograph of the kidney of Hemichromisfasciatus sampled from mainland and maritime areas, western part of Ebrie Lagoon (Ivory Coast): Ml Melanomacrophage; N focal area of necrosis; Hrhaemorrhage; V Vacuolar of tissue; F Fibrosis. (Hématoxylin- Eosin. X 400)

4. Discussion

Mean concentrations in fish organs

The concentration of heavy metals in organs of fish showed that the aquatic environment is polluted (Farkas et al., 2000).The high concentration of heavy metals observed in *Hemichromis. fasciatus*result to its positionin food chain and the diet. Indeed, several authors have reported that *H. fasciatus* is a terminal consumer and feeds on juveniles of fish, shrimps and crabs and other crustaceans that are at the top of the food chain(Youssao et al., 2011; Blahoua et al. 2017).Results show that the continental area is more polluted than the maritime area. This difference is probably due to the direct influence of river waters charged with particles from intense agricultural activities on the watershed (Coulibaly et al., 2012).

Human health risk assessment

THQ value proposed by USA EPA is an integrated risk index by comparing the ingestion amount of a pollutant has been widely used in the risk assessment of metals in contaminated foods (Storelli, 2008).THQ values in the present study were less than 1 for all individual heavy metal (Hg, Pb, Cd, As) in *Hemichromisfasciatus* and *Tilapia guineensis x Tilapia zillii* collected in the both mainland and maritime areas. These values of THQ indicate no noncarcinogenic health risk from ingestion of a single heavy metal through consumption of these fishes. This suggested that the potential health risk to local residents should be stressed.

The THQ values found in this study were similar to those of Orosun et al. (2016) and Alipour et al**.**(2018).These authors found THQ values for heavy metals less than 1 associated of the tilapia fish consumption collected respectively from River Gongola, Northeastern Nigeria and from Gorgan Bay, Iran. The THQ for heavy metals decreased in the following order: Hg> As > Cd >Pb. The potential health risk for Pb was the lowest. According to Genc et al. (2018), the low potential health risk for a metal may be ascribed to its higher oral reference dose.

Results show that the HI of metals for species exceeding 1, except HI =0. 92 of metals for *Tilapia guineensis x Tilapia zillii* collected in maritime. HI exceeding 1 indicates that the metals are toxic and present a hazard to human health (Li et al., 2013). Indeed, Hallenbeck,(1993) reported that exposure to two or more pollutants may result in additive or interactive effects.The potential health risk by *H.fasciatus* consumption was greater than Hybrid tilapia consumption.The discrepancy of HI for fish inhabiting in the both areas may be due to *H. fasciatus* species accumulates more than its heavy metals in organs the *T. zillii* × *T. guineensis* hybrid.

Histopathological effects

Histology has been used as a test forevaluating toxic effects of water pollutants in fish (Abarghoei et al., 2016; Mustafa et al.,2017; Kaur etal., 2018).Histopathological changes are used as biomarkers to evaluate the overall health of fish exposed to contaminants (Adams, 2002).In the present study, histopathological changes of liver and kidney of the fish species were studied. The important number of contaminatedorgans in *H. fasciatus* can be depending to the feeder strategies. Blahoua et al. (2017) reported that this fish species has an omnivorous diet.

The organ most associated with the detoxification andbiotransformation process is the liver, and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water (Van der Oost et al.,2003).The pathological alterations of the *Hemichromisfasciatus* and *Tilapia guineensis x Tilapia zillii* liver included focal areas of necrosis, cells hepatic degeneration,hemorrhage, fibrosis and vacuolar degeneration.These hepatic lesions observed might probably be due to the primary function of the liver in the metabolism and excretion of toxicants where some morphological changes do occur in the process (Rocha *et al.*1999).According to Manahan (1991), the occurrence of necrosis is also a consequence of enzymatic inhibition, damages in the cellular membrane integrity, and disturbances in the synthesis of proteins and carbohydrate metabolism. However, hepatic vacuolations may be due to lipid and/or glycogen deposition suggestive of metabolic disorders as a consequence of the exposure to toxic agents (Pacheco et al., 2002). Regarding the observed haemorrhage in the tissue hepatic, its might be due to the toxic damage of the liver fish exposed to heavy metals.Ibrahim et al. *(*2005) and Tayel et al.(2008) noticed the similar observations with *Tilapia zillii* and *Clariasgariepinus* liver inhabiting in Nil exposed to different pollutants. In addition, Accumulation of haemosidrin and pycknosis have been observed in *Tilapia guineensis* x *Tilapia zillii* liver. Tayel et al. (2008) rapported that abnormal accumulation of haemosidrin in liver cells may be due to rapid and continuous destruction of erythrocytes with increased hemolysis and damage of the iron metabolism. Similar alterations have been observed in the liver of *Siganusrivulatus* living along the Red Sea Coast, Sudan (Mohammed et al., 2016).These changes may be attributed to the direct toxic effects of pollutants on hepatocytes (Salim, 2015). The dilation and congestion in blood sinusoids and sever deposition of hemosiderin pigments were also observed by Ibrahim et al. (2019) in liver of *Oreochromisniloticus* from sewage water in El-kharja (Egypt) polluted by heavy metals accumulation.

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and erythropoieses (Iqbal et al., 2004). However, it is one of the first organs to be affected by contaminants in the water (Thophonet al., 2003).

In our study, several histological lesions were observed in the Hybrid tilapia and *H.fasciatus* kidney. hemorrhage. These observations agree with those of Abalaka, (2015) in kidney of *Auchenoglanisoccidentalis* from Tiga Dan, Nigéria. According to this author, tubular vacuolations in the kidney of the sampled fish were indicative of fatty degenerative changes due to metabolic disorders. Atrophy and inflammatory cell aggregates were also observed in *Tilapia guineensis* x *Tilapia zillii* kidney. In contrast, melanomacrophages aggregates in the space of Bowman's capsule were observed in *Hemichromisfasciatus* kidney. Similar pathology found by Ruqaya et al. (2013) and Brraich et al. (2017) in the kidney of *Cyprinuscarpio* and *Labeorohita* exposed to heavy metals. According to Brraich et al. (2017), the lesions in the nephron and haemopoeitic tissues suggests impaired osmotic and ionic regulation due to altered metabolic activity caused by interaction between the heavy metals and renal tissue.

5. Conclusion

This study concludes that there are significant health risks associated with consumption of *Tilapia guineensis* x *Tilapia zillii* and in *Hemichromisfasciatus* from mainland and maritime areas. Severe histopathological lesions and cellular alterations were observed in major target organs (liver, kidney) of these two fish species which could be attributed to the significant accumulation of heavy metals in these tissues. The species of *Hemichromisfasciatus* presented a wide damage and an important organs infected.Histopathological alterations inthe tissues contribute to the death of the fish species in western part of the Ebrie lagoon.

6. Acknowledgements

The authors express their sincere gratitude to the Council of Interprofessional Funds for Agricultural Research (FIRCA) for their financial support.

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