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Biological impact of in-situ exposure of catfish (*Clarias gariepinus* Burchell, 1822) to agricultural pesticide residues in the Tabalak pond

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ABSTRACT

The Tabalak pond is contaminated by residues of agricultural pesticides, used by market gardeners. The present study aims to evaluate the ecotoxicological impact of this contamination on the ichthyofauna of this pond from growth parameters, biometric indices and organs histopathology. An in-situ fish cage experiment was used, compared to controls placed elsewhere and fish in the open water of the pond. Thus, juveniles of *Clarias gariepinus* were raised in tanks (control) and Tabalak pond for 120 days. The results showed that the survival and growth of the fish were better at the control site than the Tabalak site. The survival rate (SR) was 93.75% for the control, compared to 83.13% at the Tabalak site. The mean final weight (Mfw) was 148.71 g compared to 138.47 g, the weight gain (Wg) was 133.52 g compared to 123.45 g and finally the specific growth rate (SGR) was 1.9% compared to 1.85% for the control and Tabalak sites respectively. Semi-quantitative evaluation of organ tissues showed slight alterations in the organs of the control fish and moderate alterations in the organs of the caged and open water fish. Some of these histological alterations may be detrimental to certain vital functions of these fishes.

Keywords: Pesticides, Tabalak pond, *Clarias gariepinus*, Biomarkers, Growth parameters, Histopathology.

INTRODUCTION

The introduction of undesirable substances into the water body causes changes in the physical, chemical and biological characteristics of the aquatic system that lead to ecological imbalance (Qadri and Bhat, 2020). Among these substances, we can note mineral pollutants (metallic trace elements, nitrogenous and phosphate nutrients, etc.) and organic pollutants (polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, organic pesticides, etc.).

Pesticides are among the most dangerous pollutants that cause great harm to animals in the aquatic environment, especially fish (Al-Otaibi et al., 2019). A large proportion of them have bioaccumulation and biomagnification capacities, modifying physico-chemical parameters, thus inducing modifications in the structure and functioning

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down to the cellular level of the organisms in the environment where they have been introduced. With the exception of highly volatile compounds, most of these chemical substances introduced into the environment end up invading surface waters in the medium term (Hong et al., 2019). Their introduction into aquatic environment occurs through various mechanisms (runoff, drift, erosion, volatilization, hypodermic transfer), particularly in areas close to their use. In order to meet the need to assess the biological impact of this contamination, biomarkers are increasingly used (Gonçales et al., 2021). In aquatic organisms, biomarkers are used to provide early warning signals of exposure or effects of specific pollutants or classes of pollutants (Van der oost et al., 2003). They are broadly defined as any biological response of an organism to exposure to environmental chemicals or their toxic effects (Monteiro et al., 2021). To this end, fishes are excellent experimental models for aquatic ecotoxicological assessments (Hong et al., 2019). They are sensitive to pollution, they occupy different trophic levels, they have a well-developed osmoregulatory, endocrine, nervous and immune system, and above all they are at the end of the food chain in aquatic environments and constitute an important food of source for humans (Song et al., 2012; Ribeiro et al., 2013; Imorou Toko et al., 2014). Some biomarkers such as growth parameters, biometric indices and histopathological alterations, although non-specific, have been shown to be significant indicators for assessing ecosystem health. Gills, kidneys and liver are responsible for vital functions such as respiration, excretion, accumulation and transformation of xenobiotics, regulation of extracellular fluid, as well as acid-base balance in fish, the identification of alterations constitute unique information on environmental health (Maria et al., 2009; Al-Otaib et al., 2019). These biomarkers have been widely used in studies assessing the degree of exposure of aquatic environment to pesticides (Imorou Toko et al., 2018; Agbohessi et al., 2021; Shah and Parveen, 2022).

Around the Tabalak waterhole, irrigated crops of onion, sweet potato, legumes, cassava, fruit trees, etc.... offer many financial benefits to producers. Seven (7) sites are exploited around the waterhole (Amadou, 2020). An area of about 954.44 ha is exploited. There is a high use of chemical pesticides to increase yields and some of these chemicals applied are prohibited (Comdeks, 2013; Moumouni et al., 2019).

Nine (9) pesticide molecules were detected and quantified in the Tabalak pond, notably in sediments and fish at high levels, some exceeding environmental quality standard. They are also the major pollutants in this ecosystem (Youchaou Tawayé, 2022).

This study proposes to study the ecotoxicological impact of pesticide contamination on the ichthyofauna of the Tabalak pond, Specifically *Clarias gariepinus*, through biomarkers, in particular growth parameters, organs histopathology.

MATERIALS AND METHODS

Study sites

The study was conducted at the Tabalak pond and an aquaculture farm in Niamey, taken as a reference site.

The Tabalak pond is a body of water located in the Tahoua region, where market gardening activities with high pesticide consumption are carried out in its vicinity. This pond is contaminated with agricultural pesticide residues, particularly in its sedimentary and biological compartment. Residual pesticide levels are reported by Youchaou Tawayé (2022).

The climate at both sites is semi-arid with high variability in rainfall. The average rainfall is around 500 mm per year (PDC, 2014; Issiaka et al., 2018). Temperatures can drop to values between 8 and 10°C in the cold season and rise above 40°C in the hot season in Tabalak. It varies between 23 and 36°C in Niamey (Boubacar et al., 2020).

Biological material

The species used is *Clarias gariepinus* Burchell, 1822. It belongs to the phylum Chordatae, order Siluriformes, family Clariidae, genus *Clarias*. This fish has a wide distribution in Africa, especially in its western part (Yalcin et al., 2001). It is an elongated and cylindrical fish, often dark grey or black in colour. It has a flat, bony head and a large terminal mouth with four pairs of barbels. *C. gariepinus* prefers fresh, calm water, lakes, streams, rivers and swamps (Okonkwo et al., 2020).

The choice of this species lies in the fact that pesticide residues were detected in the sediment and Clarias being a benthic species. But also, according to Issiaka et al. (2018) *Clarias gariepinus* is one of the species almost present in freshwater environments in Niger; which makes it one of the representative species of fish living in aquatic biotopes in Niger and therefore widely available.

Experimental design

The experimental approach used consisted of in situ caging of fish in the Tabalak pond. The cage, made from metal bars, had the following characteristics: 4 m long, 2.5 m wide and 1.7 m high. It was covered on all sides with a 1 cm mesh net to prevent the fishes from escaping. The control system, consisting of two ponds, was set up in an aquaculture farm in Niamey. In the pond, the metal bar cage was placed at the bottom to allow the fishes access to the sediment and to keep 20 cm at the surface. For the controls, two concrete ponds of 4 m length and 1 m width were used.

Juveniles with an average weight of 15 g and an average total length of 12 cm were purchased from Amadou Nouhou Salamat establishment in Maradi. These fishes were packaged for transport from the place of purchase to the experimental sites in 25 L containers with an opening on one side to allow oxygenation. Two batches were made up, one for the Tabalak site and the other for the Niamey site.

The stocking was carried out in November with 160 individuals per treatment: the fry fish batch in the cage constitutes the treatment (T1) and the second batch considered as control (T0) is reared in concrete basins. A third group, (T2), was added to these two groups and consisted of the pond native fish for the histological study. These fishes were purchased live from fishermen.

The stocked fishes were fed to apparent satiation twice a day throughout the experimental phase (120 days). Granules for Clarias “Vital fish feed ®” of 2, 4 and 6 mm were used.

Before setting, individual measurements of the weight and total length of the fishes were carried out, using a scale and graded ruler on a sample of 30 individuals.

Monitoring of the physico-chemical parameters of the water

During the experiment, the temperature and pH of the water were measured every three days using the Consort C5020 multi-parameter. The conductivity was also measured with this device. Dissolved oxygen was measured using the ODO 200YSI oximeter. Other physico-chemical parameters of the water at the experimental sites were also analysed: nitrates, nitrites and ammonium.

Calculation of survival and growth parameters

The survival rate is the number of fish alive after the specified time interval (rearing time). It is obtained according to the following formula:

$$SR(\%) = \frac{Fn}{In} \times 100$$

SR = Survival rate, Fn = Final number, In= Initial number.

The average initial weight (Aiw) is calculated as the ratio of the initial biomass (Ib) to the initial number (In) of fish.

$$Aiw\ (g) = \frac{Ib}{In}$$

The final average weight (Afw) is calculated as the ratio of the final biomass (Fb) to the final number of fish (Fn):

$$Afw(g) = \frac{Fb}{Fn}$$

Weight gain (Wg) is the weight gained by individuals during rearing compared to their initial weight:

$$Wg(g) = Afw - Aiw$$

The specific growth rate (SGR) gives the instantaneous rate of growth of the fish and is obtained according to the following formula:

$$SGR(\%/jours) = \frac{[\ln Afw - \ln Aiw]}{Dr} \times 100$$

Dr= the duration of rearing.

Calculation of biometric indices

The condition factor CF (%) reveals the physiological state of the fish and is obtained by dividing the body weight of a fish (W) by its total length (TL) cube.

$$CF(\%) = \frac{P}{TL^3} \times 100$$

Two organo-somatic indices were calculated, liver (HIS) and kidney (RIS), these indices are relationships between organ weight and fish weight.

$$HIS(\%) = \frac{Lw}{Fw} \times 100$$

HIS= hepato-somatic index, Lw = liver weight;

$$IRS(\%) = \frac{Kw}{Fw} \times 100$$

RSI= Renato-Somatic Index; Kw= Kidney Weight

Histological analysis of organs

After the measurement of the different morphometric parameters, the fish were euthanized and dissected. The liver and kidney were removed and weighed for the calculation of organo-somatic indices. Immediately afterwards biopsies of these organs and those of the gills were taken, fixed in 10% buffered formalin and sent to the laboratory.

Histological sections were taken in the histo-embryology and cytogenetics laboratory of the Faculty of Health Sciences of the Abdou Moumouni University. The classical method (fixation, dehydration and clearing, paraffin embedding, sectioning and staining of the sections) was used. The slides were stained with haematoxylin and eosin. Finally, the slides were covered with coverslips in a set up with Canadian Balsam. The slides were read using a Leica DM500 light microscope.

Histopathological assessment of the tissues of the organs collected was done using the standard semi-quantitative assessment tools of Bernet et al. (1999), modified by Van Dyk et al.

Alterations were observed and recorded according to the five reaction patterns described by Bernet et al. (1999):

- Reaction pattern 1 (rp1): circulatory disorders (CD), which arise from a pathological state of blood and tissue fluid circulation, apart from exudations. The alterations considered by this scheme are: (1) haemorrhage/hyperaemia/aneurysm and (2) intercellular oedema;

- Reaction pattern 2 (rp2): regressive changes (RC), which are processes that end in functional reduction or loss of the organ. The alterations involved are: (1) structural and architectural alterations, (2) plasma alteration, (3) deposition, (4) nuclear alterations, (5) atrophy and (6) necrosis;

- Reaction pattern 3 (rp3): progressive changes, these are processes that lead to an increase in cell or tissue activity. These are: (1) hypertrophy, (2) hyperplasia;

- Reaction Pattern 4: Inflammation (I), inflammatory changes are often associated with processes belonging to other reaction patterns (e.g. oedema). Therefore, it is often difficult to attribute inflammatory changes to a single reaction pattern. The authors therefore use the term inflammation in a very strict sense: (1) exudates, (2) activation of the reticuloendothelial system and (3) infiltration;

- Reaction pattern 5 (rp 5): tumors (T), which can be separated into two classes, (1) benign tumors and (2) malignant tumors.

Still based on the protocol of Bernet et al. (1999), an assessment of the lesions of the organs studied was made according to the following formula:

$$Iorg\ rp = \sum_{alt} (a\ org\ rp\ alt \times w\ org\ rp\ alt)$$

Where rp = type of alteration, a = occurrence score and w = importance factor.

For all alterations, the occurrence score (a) was multiplied by the importance factor (w) to find the organ response index (Iorg rp).

The occurrence score indicated the extent of the impairment. Four values are attributable with the possibility of considering intermediate values: (0) absent (unchanged), (2) present (low occurrence), (4) present in moderate quantity (moderate occurrence) and (6) severe occurrence (diffuse lesion).

The importance factor signified the degree of reversibility of each alteration: (1) minimal pathological importance (easily reversible), (2) moderate importance (reversible after disappearance of the pollutant) and (3) high pathological importance (irreversible leading to partial loss of organ function).

The organ indices obtained were assessed according to the classification made by Zimmerli et al. (2007), modified by Van Dyk et al.:

- Class 1 (index < 10), tissue with slight histological alterations;
- Class 2 (index 10-25), tissue with moderate histological changes;
- Class 3 (index 26-35), pronounced alteration of the tissue;
- Class 4 (index > 35), severe tissue damage.

Finally, the general health status of the fish based on the occurrence of histological alterations observed in all organs, total index (Tot-I) was calculated according to the formula:

$$Tot - I = \sum_{org}\sum_{rp}\sum_{alt}(a\ org\ rp\ alt \times w\ org\ rp\ alt)$$

The prevalence of alterations was calculated according to the formula:

$$P\ (\%) = \frac{NA}{TNE} \times 100$$

NA= Number of individuals with an alteration; TNE= Total number of individuals examined.

Data processing

The data were entered into the Excel 2016 spreadsheet. For the growth parameters descriptive statistics were applied. The results of the different observations were grouped in tables. For histological parameters, the data were processed using Minitab 16 software. The Ryan-joiner test, Bartlett's test and Levene's test were used for normality and equality of variances respectively. Data that met the criteria for normality and homoscedasticity were subjected to a one-factor ANOVA followed by Tukey's test for comparison of means, to locate the difference, where it existed. Data that did not meet these conditions were tested using the alternative non-parametric Kurskal-wallis test.

RESULTS

Water quality of the breeding sites

The physico-chemical characteristics of the experimental sites are given in Table 1. The analysis of this table shows that the two sites have relatively similar characteristics apart from conductivity, pH and ammonium. The pH values (7.26 ± 0.20 for the controls and 7.24 ± 0.31 at Tabalak) show neutral, moderately mineralized waters according to the conductivity results 715 ± 43.59 µS/Cm and 518.75 ± 323.50 µS/Cm respectively.

Oxygen levels were 4.63 ± 2.15 mg/L and 7.7 ± 0.0 mg/L; ammonium levels were 4.18 ± 0.31 mg/L and 1.91 ± 0.79 mg/L. As for nitrate levels, they were 1.8 ± 0.13 mg/L and 1.61 ± 0.06 mg/L; nitrite levels were 1.65 ± 0.2 mg/L and 1.64 ± 0.09 mg/L for the controls and fish caged in Tabalak respectively.

Influence of contamination on survival and growth parameters

The results relating to these observations are grouped in Table 2. The analysis of this table shows that, fish survival and growth was better at the control (uncontaminated site) compared to the Tabalak site (contaminated pond). The survival rate was 93.75% for the controls, compared to 83.125% at the Tabalak site. The average final weight was 148.71 g compared to 138.47 g, the weight gain was 133.52 g compared to 123.45 g and finally the specific growth rate was 1.9% compared to 1.85% for the control and Tabalak site respectively.

Histology of the organs

Types of alterations according to organs and their relative prevalence

Liver

Table 3 presents the percentages of fish by different types of alterations found in the liver. A total of 12 types of alterations were observed in the fish liver. Among these alterations, we found disorders related to the circulation of blood (haemorrhages of the veins and central organs, congestion of the sinusoids), five types of regressive alterations (structural and architectural alteration, vacuolization, plasma alteration, deposition, nuclear alteration and necrosis), two types of progressive alterations (hypertrophy and hyperplasia) and finally three types of inflammatory alterations (exudate, melanomacrophage aggregation and leucocyte infiltration). These alterations were present everywhere, but were mostly found more acutely in pond fish (open water and caged fish). Leukocyte infiltration and circulation disorders were the most frequent alterations. They were present in more than 60% of the individuals from the pond fish (native and caged) and in the controls.

Gills

Two progressive alterations (hypertrophy and hyperplasia), three inflammatory alterations (exudate, melanomacrophage aggregation and Leukocyte infiltration), one regressive alteration (vacuolisation) and circulatory disorders (haemorrhage and aneurism) were detected in the gills. The prevalence of individuals with these alterations is shown in Table 4. Few lesions were found in the control fish compared to the other treatments. For all the alterations found, the fish in the pond were the most affected: 96.66% of the fish in open water and 90% of the caged fish compared to 40% of the control fish for respiratory disorders; 83.33% of the fish were affected by hyperplasia in the pond compared to 60% for the controls; 80% and 46.66% respectively in the caged and open water fish for exudates, compared to 36.66% for the controls; 90%, 73.35% for leucocyte infiltration in the open water and caged fish compared to 30% for the controls.

Kidneys

The histological alterations identified in the kidneys and their prevalence are presented in table 5. Among the alterations detected in the kidneys, four were regressive (structural and architectural alterations, vacuolization, deposition, necrosis), one progressive (hyperplasia), three inflammatory (exudate, melanomacrophage aggregation, leukocyte infiltration) and one circulatory (haemorrhage). Compared to the other treatments, the controls showed fewer alterations of histopathological types with four types identified. Leukocyte infiltration was the most frequent alteration (86.66% of native pond fish, 66.66% of controls and 63.33% of caged fish), followed by circulatory disorders (80% of native pond fish) and finally exudates (63.33% of caged fish).

Average and total indices according to treatments

The results of the semi-quantitative assessment of organ tissues (liver, gills and kidneys) are shown in Table 6. The organ classification indices place the control treatment in class 1 (index < 10) indicating slight alterations and the other treatments in class 2 (index 10-25) indicating moderate histological alterations.

The comparison of the means showed that the alteration on the organs of fishes in Tabalak pond (caged and native) are higher than the controls. For the liver and gills, the means of the alteration indices were not statistically different between caged and native fish; rather these two treatments showed a significant difference with the controls.

On the other hand, differences were observed for the kidney, an organ in which the native fish were more affected than the other groups, which also showed a significant difference between them. The index of alterations in the caged kidneys was significantly higher than that of the controls. The total indices indicating the general health of the fish show a similar trend to that observed in the kidneys.

Variation in fish biometric indices

Table 7 shows the biometric indices of the pond fish (caged and native) compared to the controls. The analysis of this table shows that, the condition and hepato-somatic indices revealed significant differences between the three treatments. The condition factor was higher in caged fish (0.752%) followed by native pond fish (0.683%). The hepato-somatic index was also higher in the control fish (1.565%) followed by the caged fish (0.904%). However, no significant difference was found between the control treatments (0.643%) and the caged fish (0.681%), which on the other hand showed a significant difference with the native fish (0.408%) for the renato-somatic index.

Table 1: Physico-chemical parameters of the water at the experimental sites.

Area	Temperature (°C)	pH	Dissolved oxygen (mg/L)	Electric conductivity (µS/Cm)	Ammonium (mg/L N-NH_4)	Nitrates (mg/L N-NO_3)	Nitrites (mg/L N-NO_2)
Control	22,13 ± 2,20	7,26 ± 0,20	4,63 ± 2,15	715 ± 43,59	4,18 ± 0,31	1,8 ± 0,13	1,65 ± 0,2
Tabalak	22,46 ± 3,52	7,24 ± 0,31	7,7 ± 0,0	518,75 ± 323,50	1,91 ± 0,79	1,61 ± 0,06	1,64 ± 0,09

Table 2: Variation of survival and growth parameters according to treatment.

Parameter	**Area**	
	Control	**Tabalak**
SR - survival rate (%)	93,75	83,125
Aiw - average initial weight (g)	15,02	15,19
Afw - average final weight (g)	148,71	138,47
Wg - weight gain (g)	133,52	123,45
SGR - specific growth rate (%)	1,9	1,85

Table 3: Alterations identified in the liver and their prevalence.

Treatment	Circulatory distorbances	Architechtutral and Structural alteration	Vacuolization	Plasma alteration	Deposit	Nuclear alteration	Nécrosis	Hypertrophy	Hyperplasia	Exsudat	Melanomacrophage Agregation	Leukocyte infiltration
LT0	66,6	-	16,6	6,6	3,3	13,3	13,3	3,3	-	36,6	36,6	70
LT1	73,3	23,3	56,6	26,6	-	20	60	16,6	3,3	43,3	33,3	80
LT2	80	13,3	60	23,6	-	16,6	56,6	6,6	-	46,6	83,3	90

LT: liver in treatment

Table 4: Alterations identified in the gills and their prevalence.

Treatment	Circulatory distortance	Vacuolization	Hypertrophy	Hyperplasia	Exsudat	Melanomacrophage Agregation	Leukocyte infiltration
GT0	40			60	36,66		30
GT1	90	43,33		83,33	80	10	73,35
GT2	96,66	36,66	23,33	83,33	46,66	10	90

GT: gill in treatment

Table 5: Alterations identified in the kidney and their prevalence.

Treatement	Circulatory distorbances	Architechtutral and Structural alteration	Vacuolization	Deposit	Necrosis	Hyperplasia	Exsudat	Melanomacrophage Agregation	Leukocyte infiltration
KT0	33,33						53,33	50	66,66
KT1	36,66	33,33	3,33	50	13,33	13,33	63,33	53,33	63,33
KT2	80	43,33	6,66	20	63,3	16,66	36,66	36,66	86,66

KT: kidney in treatment

Table 6: Average values of organ indices and total index.

Treatment	Liver index	Gill index	Kidney Index	Total Index
T0	9,733±6,96 b	9,2±5,08 b	6,533±2,91 c	25,47±11,48 c
T1	21,367±9,67 a	17,733±5,9 a	15,1±7,77 b	54,2±11,91 b
T2	23,533±6,76 a	18,4±4,88 a	21,33±6,41 a	63,27±10 a
Probability	0,000	0,000	0,000	0,000

The means assigned with the same letter are not significantly different at the 5% threshold.

Table 7: Mean values of biometric indices.

Treatment	CF	HIS(%)	RIS (%)
T0	0,598±0,05 c	1,565±0,42 a	0,643±0,20 a
T1	0,752±0,1 a	0,904±0,30 b	0,681±0,36 a
T2	0,683±0,09 b	0,408±0,05 c	0,408±0,09 b
Probability	0,000	0,000	0,000

Means with the same letters are not significantly different at the 5% level. CF: condition factor, HIS: hepathosomatic index, RIS: Renato-somatic index

DISCUSSION

According to the synthesis of Géoffroy et al. (2019), the values obtained are within the limits tolerated by *Clarias gariepinus*, notably from 8 to 35°C for temperature; between 6 and 9 for pH. This species adapts well to low dissolved oxygen levels ranging from 6.5 to 8 mg/l or even 3.5 mg/l (Eding and Kamstra, 2001; Brougher et al., 2005). The same applies to nitrates, for which the acceptable levels for this species are between 0.2 and 10 mg/L. As for nitrite levels, they are respectively 1.65 ± 0.2 and 1.64 ± 0.09 mg/L at the control and Tabalak sites for a limit that must not exceed 0.3 mg/L. The ammonium standards are between 0.01 and 1.15 mg/L for values measured in the order of 1.91 ± 0.79 mg/L at Tabalak and 4.18 ± 0.31 mg/L at the control site. This study highlights the effect of contamination of the Tabalak pond by agricultural pesticide residues. Physiological and growth performance as well as histopathology are biomarkers used in the laboratory as well as in the wild to assess the biological effects of pesticides on organisms (Ariweriokuma et al., 2011; Odo et al., 2015; Imorou Toko et al., 2018, Al-Otaib et al., 2019, Mourya et al., 2019).

Fish caged under these conditions had a lower survival rate than the control. It is noted that fish mortality at the treatment level is mostly recorded at the beginning of the experiment, which could be attributed to handling stress (water changes) for the controls. In the fish reared in the pond, this mortality could be due to the chemical state of the environment, in particular a manifestation of the acute toxicity of the pesticide cocktail, especially as the pesticide residues were detected mainly in the sediments of this pond and *Clarias gariepinus* is a fish that lives close to the bottom of the water. The other growth parameters showed a similar trend in survival rate. Average final weight, weight gain and specific growth rate were also better in controls. The same findings were noted in other studies that compared the performance of fish in contaminated environment to those with little or no agricultural pesticide contamination (Imorou Toko et al., 2018; Pèlèbè et al., 2020). These results could indicate a better physiological state and nutritional assimilation of the control fish compared to those caged in the pond. Pesticides are known to induce changes in feeding behaviour, food conversion efficiency, energy metabolism (Gandar, 2015). However, the condition factor of caged fish and fish living in open water were higher than those of controls. These results can only be explained by a probable onset of sexual maturity in the control fish. Conversely, the regression of the hepatosomatic index of fish at the pond level could be explained by energy metabolism (Zhelev et al., 2015), the higher value of this index in control fish could be attributed to the production of vitelogenin, a protein synthesized by the liver involved in ovarian maturation, the main precursor protein of the egg yolk. Sexual maturity is cited as a factor in the variation of condition factor in fish (AquaPortail, 2023). According to Chikou et al. (2008) and Niare et al. (2012), reserves are invested in the development of sexual products. The observed reduction of the renato-somatic index in open water fish may be due to the depletion of this organ. Indeed, these fish are doubly exposed through the environment and also through feeding (Thophon et al., 2003). The kidney in fish is known to excrete harmful substances such as xenobiotic metabolites and waste products from nitrogen metabolism (Hadi and Alwan, 2012).

The histological alterations found could be attributed to the degree of pesticide pollution in the pond. Histological distortions have been widely optimised as biomarkers of pollutants in fish (Naeemi et al., 2013). Indeed, studies have found histological alterations in fish organ tissues from aquatic ecosystems contaminated with agricultural pesticide residues (Agbohessi et al., 2015; Geudegba et al., 2022; Shah and Parveen, 2022). The liver showed more alterations and the indices of this organ were relatively higher, Vander Oost et al. (2003) related this phenomenon to the crucial role of the liver in the process of detoxification and biotransformation and also to its function and position in blood supply. Similar observations were made by Hadi and Alwan

(2012) on *Tilapia zillii*. The alterations observed in the liver of fish from Tabalak pond are mostly circulatory disorders (haemorrhage, sinusoidal congestion), fatty vacuolisation, necrosis and leucocytic infiltration. Zaghoul et al. (2020) made similar observations on *Clarias gariepinus* in waters impacted by pollution, due to untreated drainage, from El-Fayoum governorate in Egypt. It is clear that damage to the gills impairs gas exchange capacity which can lead to respiratory deficiencies in individuals. But also, according to Das and Mukherjee (2000) moderate alterations in the gills caused by pollutants can retard growth and affect reproduction. Aneurysm formation is related to the rupture of pillar cells or increased blood flow or even due to the direct effects of contaminants on these cells (Martinez et *al.*, 2004). The leukocyte infiltration seen in all organs could be an inflammatory response to the presence of toxic agents in the media. The melano-macrophage aggregations have detoxification and recycling functions allowing the degradation of exogenous materials (Agbohessi et al., 2015). Liebel et al. (2013) link aneurysm to a response to acute toxicity. These authors also observed hyperplasia which they linked to chronic exposure. Other histological alterations observed in the gills were haemorrhage, leucocytic infiltration and exudates. These results seemed to corroborate in order those of Gichi et al. (2014), Liebel et al. (2013) and also Nimet et al. (2018).

The kidney is the second most impacted organ by environmental quality in this study. The most frequent reactions are circulatory disorders including haemorrhage, hyaline deposition, necrosis, exudates, melanin-macrophage aggregation and infiltration. Some of these reactions such as necrosis, hyaline deposition and exudates were observed with some difference by Senarat et al. (2015) on the kidneys of *Hemibagrus filamentus* in the river Tapee in Thailand.

Water quality is being determined to confirm whether it plays a role in the alterations observed during the experiment, as lower water quality influences stress and disease in fish (Devi et al., 2017). In addition, sub-lethal concentrations can become lethal for populations facing additional stresses (Sulekha and Mercy, 2009). Most of the parameters measured are within the tolerated limits for the species, except for the levels of nitrogen compounds that exceed critical thresholds.

The latter can also promote histological and biochemical changes and affect the organisms' growth capacities (Araujo et al., 2022). Ammoniacal nitrogen absorbed by fish enters the circulatory system and affects haematological properties. Acute and chronic toxicity can be induced even at very low doses (Mercante et al., 2018). Histological alterations have been observed in fish exposed to various concentrations of ammonia nitrogen (Mustapha and Akinshola, 2016). The same is true for nitrite, Baker et al. (2022) found concentrations causing sublethal effects in several aquatic organisms at different trophic levels at levels varying between 0.02 and 1.28 mg/L. These observations could explain the results obtained, particularly the histological alterations observed in the controls, but also the lower condition factor.

Some of these reactions such as necrosis, hyaline deposition and exudates were observed with some difference by Senarat et al. (2015) on the kidneys of *Hemibagrus filamentus* in the river Tapee in Thailand. The effects on the kidneys are likely to lead to major problems with anaemia and new blood cell formation (Abubakar et al., 2019).

Conclusion

The results of this study showed that the contamination of the Tabalak pond with agricultural pesticide residues has impacts on the organisms. The growth and physiological parameters of *Clarias gariepinus* were affected. Also, these contaminants had effects on *Clarias gariepinus* liver, gills and kidneys. Histological alterations were identified indicating probable impairment of certain vital functions of these fishes. Based on these findings, measures need to be taken to limit the impact of pesticide use by vegetable growers on this ecosystem.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

YTA is the principal author of this manuscript. He designed the research protocol and participated in all phases of its implementation. ITI advised for experimental work. AB and ITI supervised the research orientation, corrected and edited the final manuscript.

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