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Histopathological disturbances in two fish species *Oreochromis niloticus* (Linnaeus 1758) and *Labeo barbus pellegrini* (Bertin et Estève 1948) from downstream mining sites in the upper Ulindi and Elila river basins, Eastern DR Congo

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ABSTRACT

The effect of intensive mining on the health status of fish living in the upper basins of the Ulindi and Elila rivers was investigated between June 2018 and March 2022 by comparing the reproductive, hepatic and gill systems of fish collected from sites upstream and downstream of the mining sites. Biometric indices and organ histopathology are biomarkers that were studied at two different trophic levels using two fish species *Oreochromis niloticus* and *Labeobarbus pellegreni*. Histological evaluation was performed on the liver, gonads and gills of both species. Water and sediment samples were analysed for total mercury (T-Hg) concentration by (atomic absorption spectrophotometry). Histological changes were assessed semi-quantitatively, and the results were graded according to the severity of the histological responses. The results showed that L. pellegrini accumulated more T-Hg than O. niloticus. The T-Hg level was higher in the organs of both species during the rainy season than during the dry season in the following order: Gonads > Liver > Gills and exceeded the WHO/FAO recommended limit (T-Hg = 1.0 mg/kg wet weight) only in the testes and gills of L. pellegrini collected at the downstream sites of the Ulindi and Elila rivers. This does not necessarily reflect the level in the carcass. Taken together, the results suggest that mercury accumulated downstream of gold mining operations is altering the health of fish populations and that L. pellegrini can be considered a sentinel species for monitoring this pollution. To protect fish health, it is recommended that certain mining practices such as the misuse of mercury be avoided throughout the Congo River basin.
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Key words: Fish Histology, River, Gold panning, Estrogenic effect, biomarkers, Mercure.

INTRODUCTION

The impact of water pollution and waterborne pollutants on human health and aquatic organisms has become a major concern and has attracted much research attention in recent decades (Marchand, van Dyk, et al., 2009). Untreated mining, industrial, agricultural and domestic effluents are the most common causes of pollution of aquatic ecosystems (Koca et al., 2005; OMS, 2019).

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Download SUPPLEMENTARY MATERIAL

Gold mining operations often result to an increase in heavy metals (such as cadmium, lead, copper, etc) exceeding the recommended limits for quality water (Mambou Ngueyep et al., 2021; Ranjbar et al., 2022; Obodai et al., 2023) It is recognized that the metabolic processes of aquatic organisms require, at low concentrations, certain so-called essential metals. However, while mercury (Hg) is included in the list of metals detected in fish, it is not considered an essential element in vertebrates and its presence, especially in its methylated form (MeHg), is considered toxic even at trace levels (Arantes, 2008; Sampajo, 2008). Recent evidence suggests that artisanal gold mining and coal burning account for about two-thirds of anthropogenic mercury emissions (Barst, 2015). Emitted mercury is introduced into the atmosphere and deposited in aquatic and terrestrial ecosystems (UNEP, 2002; WHO, 2007). In aquatic environments, mercury undergoes methylation and then readily accumulates and bio-amplifies in food webs, resulting in high concentrations in fish (Osman and Wener, 2010). Its presence in the fish body can alter certain tissues and create various physiological dysfunctions (Mc Hugh et al., 2013; Funmilayo et al., 2019). MeHg can cause a lack of coordination, loss of appetite, reduced swimming ability, and mortality in fish (Bernstssen et al., 2003; Degila et al., 2019). MeHg is also known to be teratogenic, and oxic to embryos and may also affect behaviour, growth, and reproduction, as well as affecting gonadal development or adult spawning success, or decreasing egg hatch, health, and survival in different developmental stages (Wiener et al., 2003; Chekri et al., 2016).

Metals are taken up by aquatic organisms by ton main routes, water (direct route) and (trophic route) (Sampajo, 2008; Sanou e al., 2020; Manu et al., 2020). Metal uptake requires the passage of specific biological structures such as the external lining and especially the gill epithelium for contaminants present in water, and the entire digestive tract for metals associated with particles or contained in ingested prey (Belaj & Heidi, 2020). This absorption of metals by aquatic organisms is modulated by numerous factors such as the stability of the bonds of the metal in question with the blood constituents, the vascularisation of the organs, the structure and properties of the numerous cellular barriers (capillary walls, plasma membranes, etc.), the density and accessibility of potential fixation sites or the regeneration of tissue structures (Chebli et al., 2020). The phenomenon of bioaccumulation of of heavy metals in aquatic organisms will depend on many factors, first and foremost the nature of the metallic element, depending on whether it has a biological role or not and whether it is regulated or not by the organism (N'Guessan et al., 2021). Heavy metals are naturally present in trace amounts in soils, water or sediments depending on their geological characteristics (natural soil geochemical concentrations). Natural deposition processes, for example, those related to volcanic activity, also influence their concentrations. Bioaccumulation occurs when an organism absorbs a contaminant more quickly than it eliminates it. Bioaccumulation depends on biotic factors such as life stage, age, sex, physiological condition and abiotic factors such as the degree of pollution and the season. Moreover, most of these factors interact and it is often difficult to distinguish between them. Thus, during the life cycle, bioaccumulation can vary considerably. (Djeddi et al., 2018; Khadra, 2019; Charrette, 2021).

The Eastern part of the Democratic Republic of Congo in general and the Mwenga Territory, in particular, are well known for their mining, demographic development, and agriculture as well as livestock (Cubaka et al., 2019). As a result, the freshwater catchments in this region may be negatively affected by the release of heavy metals and domestic effluent as well as the release of untreated wastewater (Musamba & Vogel, 2016b). In most of the Congolese gold mining sites, mercury (Hg) is used to a much greater extent in the processing of gold in the catchment areas and on the various riverbeds of the Congolese rivers in general and the Ulindi River in particular, a tributary of the right bank of the Congo River, which can accentuate mercury pollution of water and aquatic organisms including fish (Muhima, 2018).

However, information on the distribution and toxicological effects of this metal on organs and their vital functions is lacking to date for DRC river systems such as the Ulindi River. The numerous data available only concern the socio-economic impact of gold mining, the ichthyological diversity, and the biology of fish, as well as the physico-chemical characterization of the water in certain rivers (Rwabashi, 2016).

The gills of fish are in permanent contact with the aquatic environment. Due to their delicate structure, direct contact with water, and multiple important functions, gills are the first organ to be affected by pollutants and are therefore generally used as primary markers of aquatic pollution (Azadbakht et al., 2019a; Bernet et al., 1999; Gélinas, 2019). They have a very thin epithelium and their total surface area is considerably larger than that of the skin epithelium (Roberts, 1989), making this organ a suitable site for xenobiotic uptake (Lujić et al., 2014 Dahani et al., 2019).

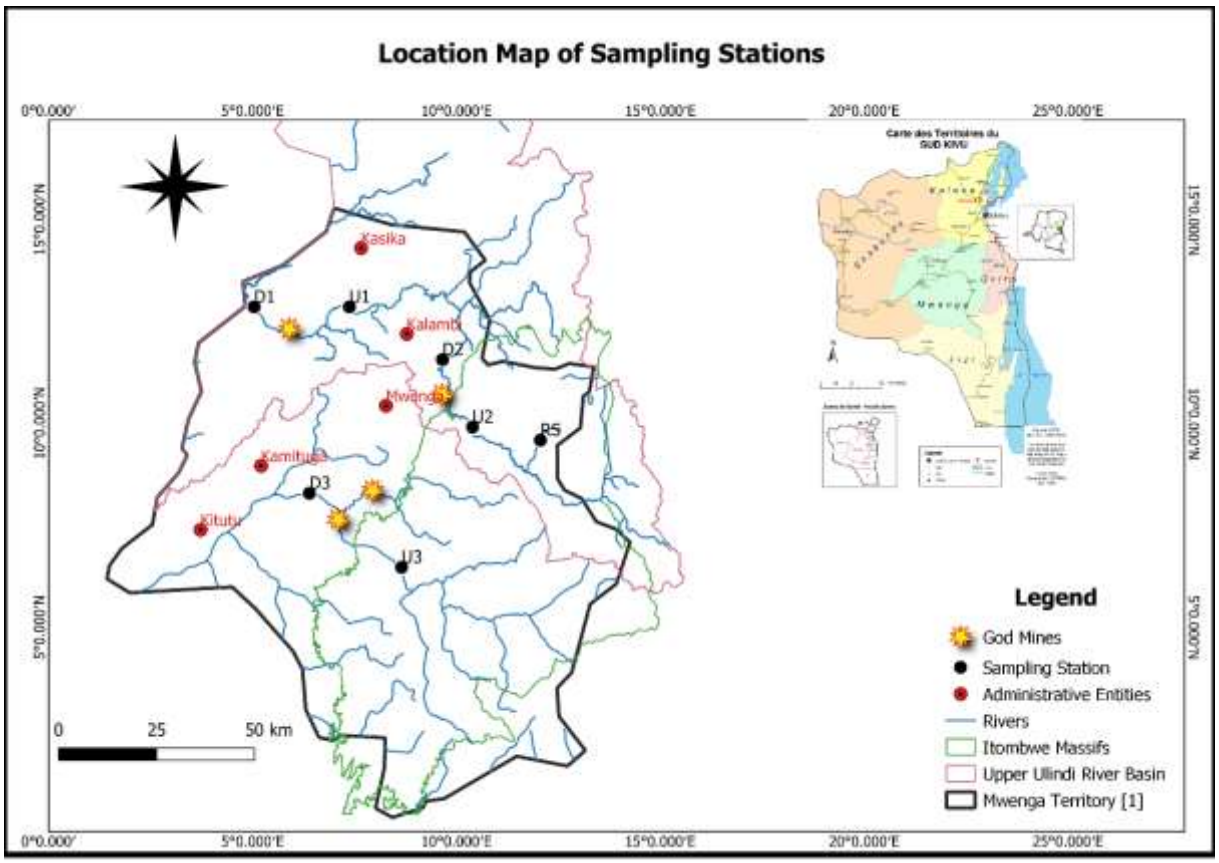

Figure 1. Location map of the different sampling stations in the Ulindi River (U1/D1-U2/D2), River (U3 and D3), and Mikyaba River (RS) of the mining sites in Mwenga territory.

The gonad is used in histology to evaluate the osteogenic effects in fish due to pollutants (Brsuslé and Solange, 1982, Louiz et al., 2018). The liver is a multifunctional organ that plays a role in the digestion and absorption of nutrients and serves in detoxification and plays an important role in the immune system (Dyk et al., 2012, Meyssan et al., 2018 Bouznada et al., 2022).

Labeobarbus pellegrini (Bertin and Estève, 1948) is present in the Ulindi River basin as a native species in contrast to *O. niloticus*, one of the introduced species in this basin (Okito et al., 2020; Kisekelwa et al., 2020). *L. pellegrini* has long been considered as a species of no commercial value in the Mwenga fisheries but it is becoming increasingly important as a fish of commercial interest in the region. Very few studies have been carried out on the life history of *L. pellegrini* compared to other African *Labeobarbus* species. For benthic organisms in particular, which are in direct contact with the sediment, a high level of contaminants in the sediment can have a great impact on their survival. Bioaccumulation and transfer through the food chain can be greatly affected by pollutants associated with sediments (Feyrouz, 2016).

Very few studies have been devoted to determining the sources and assessing the impact of pollutants on the health of fish inhabiting the aquatic environments of the province of South Kivu and more particularly the territory of Mwenga in eastern DR Congo. The numerous data available only concern the socio-economic impact of gold mining, the ichthyological diversity and biology of fish, and the Physico-chemical characterization of water in certain rivers (Nkuba et al., 2017; Nkuba et al., 2019; Mukabo et al., 2020; Kisekelwa et al., 2020; Balegamire et al., 2022; Bikubanya & Radley, 2022; Geenen et al., 2022). However, it was noted that fish is the main source of animal protein for the population living along the Ulindi River basin. From this observation, the importance of assessing the impact of pollutants on the health of fish in the upper reaches of the Ulindi, Mobale, and Mikyaba rivers in Mwenga Territory emerges; among other things, to warn local consumers of the dangers to their health, and also to address the potential effects of pollutants on the reproductive health of the local ichthyofauna. Furthermore, there are no studies to date on the histopathological assessment of the health status of fish in national waters, which are subject to heavy anthropogenic pollution.

This pioneering work aims to assess the health status of fish from mining sites in the Upper Ulindi and Elila River Basin in eastern DR Congo, using biometric indices (K, IHS, IGS) and histology of gills, liver, and gonads in *O. niloticus* and *L. pellegrini* living in this river as biomarkers.

MATERIALS AND METHODS

Location and Description of the Study Environment

This study was carried out in the Democratic Republic of Congo, in the upper basin of the Congo River (Lualaba), more precisely in the upper reaches of two of its tributaries, the Ulindi and Elila rivers in Mwenga Territory. They are located respectively between 01° 39' 44' S and 25° 48' 56'' E, and 2° 44′ 29″ S, 25° 52′ 17″ E in the Mwenga Territory of South Kivu Province, 120 km southwest of the city of Bukavu (capital of South Kivu Province). The climate of the Mwenga territory is humid tropical with a long rainy season from September to May and a short dry season from June to September. In most of the territory, the temperature varies between 21 and 37°C except in the Itombwe massifs which is a high altitude region (over 2000 m) where it is low (Ishara et al., 2022). The average annual rainfall varies between 799 mm and 2891 mm. Mwenga's hydrography is abundant with two tributaries of the Congo River. The vegetation consists of dense forests and savannahs on sandy clay soil, rich in fauna. The subsoil of this region is very rich, with minerals such as gold, cassiterite, coltan, wolframite, etc. The Ulindi and Elila rivers are right-bank tributaries of the Congo River in the Lualaba Basin and are the longest rivers in South Kivu and Maniema Provinces in the Democratic Republic of Congo with 670 and 570 km respectively (Marlier, 1954; Chuck et al., 2020). They all have their sources in the western slopes of the Mitumba Mountains at an altitude of more than 2,500 m. At this point, the mountain constitutes the watershed between the Lualaba and Tanganyika basins. The surface areas of their catchment areas are 30,240 and 30,000 km^2 respectively (De Magnée & Devroey, 1954; E. Devroey, 1957; E.-J. Devroey & Vanderlinden, 1956; Doumenge, 1998; Robert & Devroey, 1953). It drains the western flank of the Albertine Highlands, towards the Lualaba equatorial forest (Marlier, 1954). The Mobale River is a tributary of the Zalya River, which in turn is a tributary of the Elila River which, like the Ulindi River, is a tributary of the Congo River in its upper reaches, called Lualaba **(Figure-1).**

Sampling Sites

The Samples for this study were taken upstream and downstream of the mine sites in rivers belonging to two different catchment areas (Ulindi and Elila), more specifically in the Ulindi River (Kalambi section (U1 and D1) and Mwenga section (U2 and D2) and the Mobale River (U3 and D3) The Mobale River is a left bank tributary of the Zalya River, which in turn is a right bank tributary of the Elila River, located respectively in the communities of Chefferie de Basile in Mwenga Territory and the town of Kamituga in South Kivu Province in the Democratic Republic of Congo (DRC). The Ulindi River is the longest river in the South Kivu region of the DRC, rising to over 2,800 metres above sea level in the Itombwe highlands at Niakunduku, not far from Lake Lungwe in Mwenga territory. It crosses the territories of Walungu, Mwenga, and Shabunda in South Kivu, respectively the territories of Pangi and Kailo, to join the Congo River near the locality of Kowe in the territory of Punia in Maniema province (Marlier, 1954).

The stretch of the Ulindi River at Mwenga and Mobale collect drainage water from most mining, commercial and urban areas. These sites were selected based on their accessibility and expected pollution levels while being representative of the rivers in the Mwenga mining basin. In each site, two stations (upstream-downstream) were

Table-1. Geographic coordinates of the sampling sites with ST: Reference site, Upstream (U1, U2 and U3) and Downstream mining sites (D1, D2 and D3) of the Ulindi and Elila rivers, respectively.

Locality	River Basin	River/ Station	Latitude	Longitude	Position / Site
Kasika	Ulindi	Mikyaba (RS)	2° 48' 7.2" S	28° 28' 40,8" E	Reference Site
Kalambi		U1	2° 56' 31.24"S	28° 30' 55.05" E	Upstream
		Ulindi	2° 57' 47.46" S	28° 28' 55.76" E	Mine Site
		D1	2°57'38.54"S	28°27'48.7"E	Downstream
Mwenga		U2	2°59'59.71"S	28°35'41.23"E	Upstream
		Ulindi	3° 1'11.76"S	28°36'44.16"E	Mine Site
		D2	3° 1'44.71"S	28°37'16.60"E	Downstream
Kamituga	Elila	U3	3° 02'44.15"S	28°12'32.71"E	Upstream
		Mobale	3°03'22.56"S	28°12'53.04"E	Mine Site
		D3	3°03'57.93"S	28°12'13.31"E	Downstream

also selected according to the location of the mining activities. It is important to note that gold mining was taking place at the time of sampling, so the gold mining pit does not constitute a migration barrier for fish between upstream and downstream.

In Figure 1, the different sampling sites are presented and the geographical coordinates, village names, and positions of each station are presented in Table 1. These sites are areas of the rivers where the affluents converge and provide sufficient organic and mineral matter, where there is a high use of mercury in gold processing, and where the slope of these rivers allows the drainage of this and other pollutants (trace metals) by leaching from the mining sites. Faecal wastewater and illegal dumping, among other things, are the main polluters of these rivers. The two rivers Ulindi and Mobale are located in the same geographical area and have similar climatic conditions to the reference site (Mikyaba River). Kasika is a logging area where gold is rarely mined. Kalambi is an area characterized by agriculture, fishing, and livestock as the main activities of its population. The effluents from these farms are the unauthorized waste disposal sites and the faecal sewage system. The sites of Mwenga and Kamituga, on the other hand, are characterized by a high population due to the presence of several mining sites. The main polluters in these two localities are also unauthorized dumping and the sewage system, mining and agricultural waste, and livestock.

Water and sediment sampling, and tissue collection for T-Hg analysis

The samples for the histopathological study were collected between June and September 2018 on the one hand and between November 2018 and August 2019 covering the dry season and the rainy season respectively.

During the 2021 dry season (July and August) or the 2022 rainy season (February and March 2022), we sampled different river matrices (water, sediment and fish liver, gonads, gills and muscles) for the following heavy metals: cadmium-Cd, tin-Sn, zinc-Zn, copper-Cu, lead-Pb, nickel-Ni, iron-Fe, chromium, and mercury-Hg (only mercury data are presented in this work due to the length of the manuscript. The results concerning the bioaccumulation of other metals are the subject of another article in progress.). The number of stations and station matrices studied is summarized in Table 1. For each station, water was sampled at 3 locations. The water collected from the different locations was pooled and filtered over 2 mm to remove organic and other larger materials. Then, 2 liters of water from each station were brought to the laboratory in a refrigerated box (4°C). Upon arrival at the laboratory, the water sample was filtered through a 125-mm Teflon mesh sieve to remove large organic particles and then through a 1.2-mm filter. These filters were used to analyze heavy metal concentrations in suspended solids, while 10 ml of the filtrate was used to quantify heavy metal concentrations in water. The filters were cleaned with 10% HNO_3 before use. For Hg analyses in water samples, acid-cleaned glass bottles (1% HNO_3) were used and filtration was performed as soon as possible with appropriate filtration equipment pretreated for trace metal analysis to avoid contamination.

Sediment samples were collected from the top layer of sediment (0-3 cm) at 3 sampling points at each station (1 in the middle of the river and 2 at the river's edge near each bank). They were placed in plastic bottles and dried at 60°C for 48 h. Prior to analyses, they were calcined at 650°C and then acidified ($HClO_4$-HF-HNO_3) at 110°C overnight before being evaporated and stored in a 5% HNO3 solution (US EPA, 2009; B. Mandiki et al., 2014). In addition to the abiotic matrices at the stations, heavy metal bioaccumulation was tested in two fish species, *O. niloticus* and *L. pellegrini* that were collected from the stations. A total of 7 stations were studied, with 10 fish (5 males-5 females) for each station per season, based on fish population size. In the laboratory, the fish were freeze-dried and digested with an acidified solution (H_2O_2-HNO_3) and then stored in nitric acid (5% HNO_3). Then, heavy metal concentrations were measured in the digested solutions using atomic absorption spectrophotometry equipped with a cold vapor system (VGA 77). Multi-element standard solutions at different concentrations (0, 0.02, 1, 5, 20, 100, and 200 ppb) were used for calibration. The coefficients of total variation of four repeated measurements ranged from 1.66 to 5.45%. Heavy metal levels in water were compared to the Belgian and international reference quality for surface water : 1, 50, 50, 200-300 µg l^{-1} and 770 ng l^{-1} for Cd, Pb, Cu, Zn and Hg, respectively (Delbeuck 2007; Reynders et al., 2008; USEPA, 2009; Rimondi et al., 2012), and those in sediment were compared to the probable effect concentrations reported by (Flück et al., 2012; S. N. M. Mandiki et al., 2014; Shanbehzadeh et al., 2014) : 4.98, 149, 128, 459 mg kg^{-1} and 1.06 mg $kg^{-1}$for Cd, Cu, Pb, Zn and Hg, respectively.

Collecting Samples For Histopathological Study

The fish were caught by the artisanal fishing technique, using a hawk net with a mesh size of 2 cm and a diameter of 5 m, and a gill net 24 to 30 cm long and 1 to 1.5 m wide. A total of 140 fish specimens (70 per species) were sampled, 5 per sex, per station and species. Before any manipulation of the fish, they were anaesthetised (180 mg of MS 222 per litre of water), and then euthanised directly by cervical dislocation. After that, sexually mature individuals of the desired species were selected (*O. niloticus* and *L. pellegrini*), the sex of each fish specimen was determined, the weights (total and eviscerated) were recorded using a KERN EG 620-3NM scale with a capacity of 620g, the total length was recorded with a Mitutoyo calliper (with an accuracy of 0.02 mm). After all these manipulations and extraction of organs for histopathological study, the rest of the fish were put in labelled polyethene bags and placed in a well-sealed

cooler and kept cold at -4°C until further toxicological analysis.

Samples of the gill (the second gill arch on the left side of each fish), liver (median section), and mature male and female gonads (median sections of the left gonads) were taken from each adult fish, weighed,d and preserved in formalin (10% VWR, Belgium) for histological analysis. The gonadosomatic (GSI) and hepatosomatic (HSI) indices as well as the condition factor (K: the ratio of fish weight to length times 100) of each fish were also calculated. For each station and each season, a sample of 26 mature male and female fish (sexually mature) with 13 specimens per sex was sampled for each of the two species considered.

Organ Collection and Tissue Processing

Fish collection and tissue sampling at each site were performed by the same research team and the same standard methodology for tissue collection, tissue processing, and histological analysis was employed. Samples of gill (the second gill arch on the left side of each fish), liver (midsection), and male and female gonads (midsections of the left gonads), fixed and preserved in a 10% buffered acetic formaldehyde solution in glass beakers and deposited in a secure environment pending analysis at Cell and tissue laboratory (CeTi Lab) of the University of Namur. Tissues were then dehydrated through a graded methanol series, clarified with toluene, and embedded in kerosene. Sections of 0.6 µm (for gills and liver) and 0.5 µm (gonads) were mounted on glass slides for staining using the first standard techniques for HES (Hematoxylin-Eosin-Safran) for all organs. Then, specific staining was done for further histological studies.

Histological Analysis

Ovary sections were stained with a trichrome (Tri-green): hematoxylin Gill III (Merck), phloxine B (0.5%, Merck)) and light green (0.5%, FLUKA) for oocyte maturation stage determination and with hematoxylin, eosin, and Safran (HES) for histological alterations. Testis and liver sections were stained with HES trichrome. All sections were examined using light microscopy with a range of magnification (10–40x). The oocyte maturation stages were estimated according to Richard and Kestemont (1996), and Agbohessi et al., (2015a, b) with some modifications:

- stage 1: Protoplasmic oocyte or oocyte with vacuole-free cytoplasm,
- stage 2: Cortical alveoli,
- stage 3: early vitellogenesis oocyte,
- stage 4: late vitellogenesis oocyte,
- stage 5: final maturation,
- stage 6: post-ovulatory stage.

The percentage of each stage was determined on 100 cells counted per ovary. Fifty diameters of vitellogenin stage (stage 4) oocytes per ovary were measured from binoculars. Only oocytes cut through the nucleus were measured. For both sexes, a qualitative histopathological assessment was performed using a multi-head Olympus light microscope using different methodological literature for identifying fish organ alterations (Almin, 2011; Genten et al., 2010; Johnson et al., 2009)Histological alterations of the gills, liver, and gonads were assessed semi-quantitatively using a protocol developed by Bernet et al. (1999) adapted by Camargo and Martinez (2007) and Van Dyk et al. (2012). The total organ index: Gi for gills, Li for liver, Ti for testis, and Oi for ovaries, was calculated according to Bernet et al. (1999) using the following formula:

$$I_{org} = \sum_{rp} \sum_{alt} (a_{org\ rp\ alt} \times \omega_{org\ rp\ alt}) \quad (1)$$

Where: org = organ; rp = reaction pattern; alt = alteration; a = score value; w = importance factor.

The indices were classified depending on the severity of the histological response using the Van Dyk et al. (2009b, 2009a) classification based on the scoring system by Zimmerli et al. (2007).

Class I (Iorg <10): Normal tissue structure with mild histological changes.
Class II (10 ≤ Iorg ≤25): Normal tissue structure with the presence of moderate histological alterations.
Class III (26 ≤ Iorg ≤35): Pronounced alteration of organ tissues.
Class IV (Iorg >35): Severe alteration of organ tissues
To compare the overall health status based on histological lesions, the total index (TI) was calculated according to Bernet et al. (1999), by summing all organ indices of an individual fish. This index has the general formula:

$$IT = \sum_{org} \sum_{rp} \sum_{alt} (a_{org\ rp\ alt} \times \omega_{org\ rp\ alt}) \quad (2)$$

For abbreviations, see the formula Organ Iorg Index. In our study, the total index (IT) for each fish was calculated by

$$IT = Go_i + L_i + G_i \quad (3)$$

Where Go_i = Gonad index (testis or ovarie index), L_i = liver index and G_i = gill index. We thus determined the average total index by sex, species and site.

In addition to the indices calculated by the extent (score value) and pathological importance (importance factor) of the lesions, another point of interest is the prevalence of histopathological features. The prevalence of each alteration was calculated as the percentage occurrence of an alteration in all fish at each site. The formula was:

Percentage prevalence of histopathological alterations = Number of fish with that alteration / Total number of fish examined x 100.

Analysis of the Data

The results were expressed as mean ± standard deviation of the mean. For each parameter studied, the data were tested separately for males and females to minimize the effect of sex for each species. Then, the differences between the means were evaluated by a three-way analysis of variance (ANOVA III) with the factor's species, season and station. The ANOVA III was followed by Tukey's comparison as a post hoc test to compare the means of each exposed site (Downstream) with those of the reference site (Upstream) for the biometric indices (K, GSI, HSI). ANOVA One-Way in addition to the ANOVA 3 was used to compare the sexes of the two species considered for the same parameters. The non-parametric Kruskal-Wallis test was used to compare histological parameters and total mercury content in each organ by the site (Reference vs Upstream vs Downstream), season and species. A p-value of 0.05 or less was considered significant. For means expressed as percentages, the data from the statistical analysis were log-transformed (log [x + 1]) for normalisation. Calculations were performed using R for Windows version 4.1.0. Microsoft Excel was also used for the construction of some graphs.

RESULTS AND DISCUSSION

Mercury Levels in Water and Sediment

Figure-2 shows the results of T-Hg levels in water and sediment of each site and season. These results showed significant differences between the upstream and downstream sites for each of the two rivers Ulindi and Mobale, and then with the reference site (Mikyaba) irrespective of the season (Kruskal-Wallis, $p < 0.05$) for both water matrices. The results showed significant seasonal fluctuations (Kruskal-Wallis, $p < 0.05$) in T-Hg levels in both water and sediment. The highest mean concentrations of T-Hg in water and sediment were $0.77.10^{-3}$ mg.L^{-1} and 0.38 mg.kg^{-1} respectively **(Figure-2).**

Mercury Levels in Fish Organs

The T-Hg accumulation in the tested organs of each of the two species considered at the sites indicated significant variation ($F= 7.124$, $P < 0.05$, Figure-3) between the sampling stations. Mercury levels varied ($F=8.413$ $p < 0.05$) between organs and values for all organs considered were higher during the rainy season than the dry season. The analysis of variance (ANOVA) showed a significant seasonal variation ($F= 6.121$ $p<0.05$) of mercury only for the liver, testes and ovaries. The order of accumulation of this metal in the organs of *O. niloticus* and *L. pellegrini* were Gonads > Liver > Gills. The average Hg values were higher in all organs of *L. pellegrini* compared to those of *O. niloticus*. Regarding the sex, the results in figure 5 show that males accumulated more mercury than females for both species together ($F=7.717$ $p <0.05$). A significant difference is noted between the upstream (U1-U3) and downstream (D1-D3) sites on the one hand and between the mining exposed and control sites for mercury measured in the organs of both species considered ($F=9.14$ $p<0.05$) This difference in accumulation between sites is recurrent in the Gonads (male and female); Liver and gills (Figure-3).

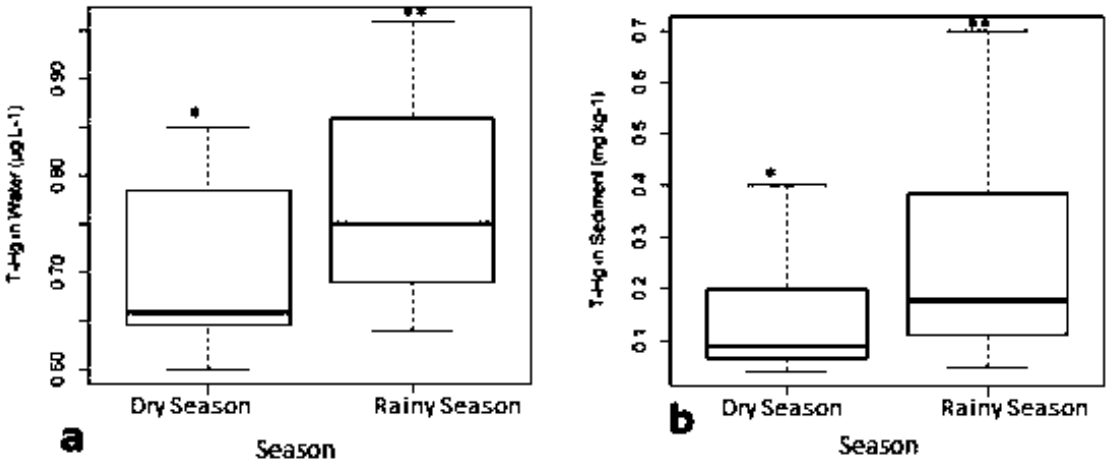

Figure-2. Variation of T-Hg levels in water (a) and sediment (b) at the upstream and downstream sampling sites of the Ulindi, Mobale, and Mikyaba River mine sites with RS (Mikyaba River reference site), Ulindi River1 (U1: Upstream and D1: Downstream), Ulindi River2 (U2: Upstream and D2: Downstream), Mobale River (U3: Upstream and D3: Downstream). * and ** indicate the significant difference between seasons ($p<0.05$).

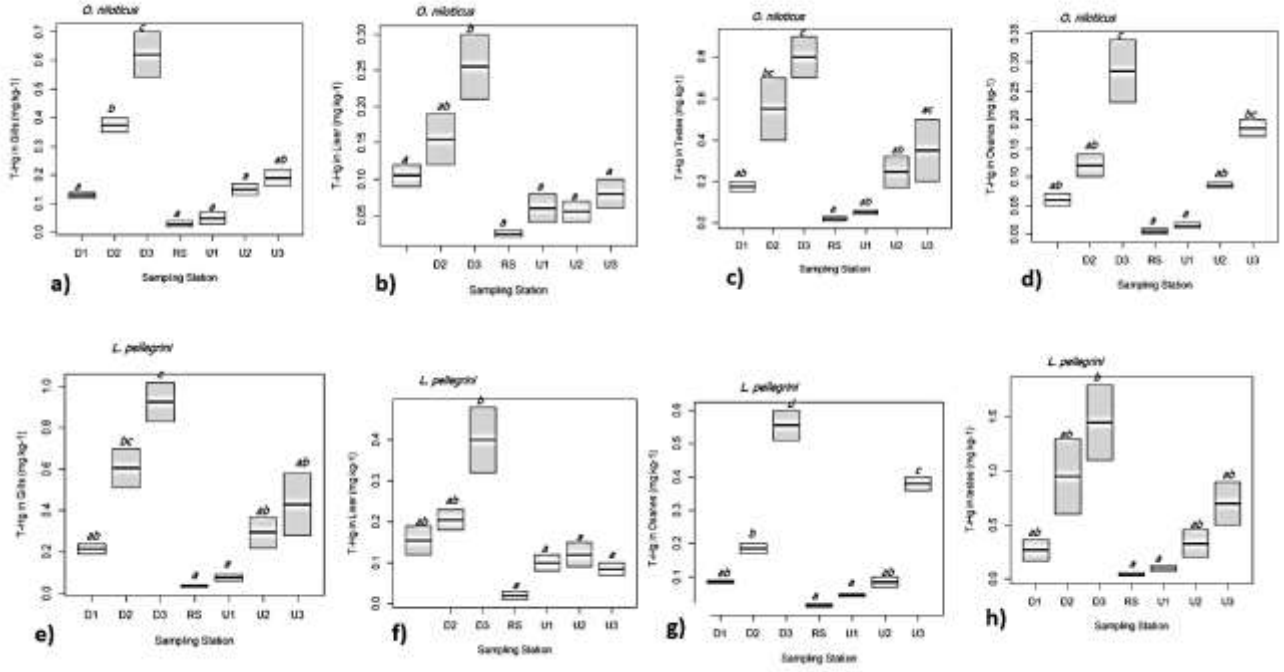

Figure-3a-h. Seasonal variation in mercury levels in gills, ovary, liver, and testes of O. niloticus (a-d) and L. pellegrini (e-h) from Ulindi River mine site. RS (Mikyaba River reference site), Ulindi River1 (U1: Upstream and D1: Downstream), Ulindi River2 (U2: Upstream and D2: Downstream), Mobale River (U3: Upstream and D3: Downstream). For each organ, different lowercase letters indicate the significant difference ($p<0.01$) between the stations.

Biometric Indices

Gonado-Somatic Index (GSI)

Analysis of Tables-2 and 3, shows a significant difference between the site ($F= 101.9$, $p<0.001$) with the highest GIS values at the reference site for both species independently of season. Similarly, individuals from the upstream site (U1-U3) showed significantly elevated values of this parameter compared to the downstream sites (D1-D3) ($t =13.562$, $p<0.001$). Compared to the reference site, the GSI results observed at the downstream sites (D1-D3) were significantly lower than those at the reference site ($t= 9.202$, $p< 0.001$), whereas for fish at the upstream sites (U1-U3), the GIS was numerically lower than those at the reference site (RS), but with no significant difference ($p> 0.05$). Species comparison for GSI revealed a significant difference between *L. pellegrini* and *O. niloticus* with *L. pellegrini* having the highest values irrespective of site and season ($F= 1964.5$, $p< 0.001$) and for both species, the GSI of females was significantly higher than those of males at all sites and in all seasons ($F=5.794$, $p<0.05$).

Hepato-Somatic Index (HSI)

As for the GSI, HSI (Table-2) varied from site to site, it was higher in females upstream than downstream in 2/3 of the sites for *O. niloticus,* the situation was different in the Mobale River (U3 < D3: $t= 2.723$ $p < 0.001$) for the same species independently of seasons. In *L. pellegrini* on the other hand, the elevated HSI upstream was only observed in Ulindi 1 (U1 > D1: $p < 0.001$) but for the other sites, the elevated HSI values were found downstream (U2 < D2, U3 < D3; $t= 3.250$ $p < 0.001$).

In males, HSI was elevated downstream in 2/3 sites: U2 < D2, U3 < D3 in *O. niloticus* regardless of season. The situation was different for *L. pellegrini* where the HSI was higher upstream than downstream in 2/3 of the sites: U1 > D1, U2 > D2 ($t = 4.752$ $p < 0.001$) for both seasons. Similarly, the HSI of the reference site (RS) was significantly higher than the Downstream (D1-D3) sites ($t= 2.714$, $p<0.05$) and numerically higher than the Upstream (U1-U3) sites but no significant difference was observed between them ($p>0.05$). A significant difference was observed between seasons with higher HSI values in the rainy season ($F= 3.914$, $p< 0.05$). The HSI of *L. pellegrini* was significantly higher than those observed in *O. niloticus* for all factors considered ($F= 77.82$, $P< 0.001$) species.

Fulton Condition Factor (K)

As with GSI and HSI, K decreased significantly from Upstream (U1-U3) to Downstream (D1-D3) ($F= 16.685$, $p<0.001$) for both species but not for some sites in *L. pellegrini* where K: D1>U1 and D3 > U3 ($F= 4.863$ $p < 0.05$). The reference site (RS) was significantly higher than the

Table 2. Seasonal and site-specific changes in Females adult *Oreochromis niloticus* and *Labeobarbus pellegrini* collected from the Ulindi and Elila river basins upstream and downstream of gold mining operations
(K: Condition Factor, RS (Reference site: Mikyaba River), Ulindi River1(U1: Upstream and D1: Downstream), Ulindi River2 (U2: Upstream and D2: Downstream), Mobale River (U3: Upstream and D3: Downstream)

Biometric indices in Females of O. niloticus and L. pellegrini						
Sites	**GSI (%)**		**HSI (%)**		**K (%)**	
	O. niloticus	***L. pellegrini***	***O. niloticus***	***L. pellegrini***	***O. niloticus***	***L. pellegrini***
	Rainy season					
RS	7.5±4.2^{aA}	9.3±3.4^{Ab}	8.1±3.2^{aA**}	10.8±1.8 ^{bA**}	2,4 ± 0,7^{Aa}	3.2 ± 0,4^{bA}
U1	6.9±2.7^{aA*}	5.3±1.3^{Ab*}	7.1±0.4^{aA}	8.9±5.4 ^{Ab*}	2,0 ± 0,4A^{aA*}	2.7 ± 0,1^{bA*}
D1	2.6±1.5^{aA**}	3.3±1.1 ^{Ab**}	4.1±1.2^{aA**}	6.3±2.5 ^{Ab**}	1,3 ± 0,5A^{a**}	2,1 ± 0,6^{bA**}
U2	8.1±3.6^{aA*}	6.1±2.4 ^{bA*}	4.5±1.9^{aA*}	8.1±3.0 ^{bA*}	1,8 ± 0,08A^{a*}	2,9 ± 0,2^{bA*}
D2	3.1±1.5^{aA**}	6.9±2.2 ^{bA*}	3.3±2.0^{aA**}	9.6±2.9 ^{bA**}	1,1 ± 0,01A^{a**}	2,0 ± 0,1^{bA**}
U3	9.1±3.6^{aA*}	8.3±1.8 ^{bA*}	4.8±1.7^{aA*}	6.3±1.2 ^{bA*}	1,7 ± 0,02^{Aa*}	2,6 ± 0,26^{bA*}
D3	2.9±1.4^{aA**}	4.5±1.0 ^{bA**}	7.8±2.8^{Ab}	9.8±1.7^{Ab}	1,0 ± 0,07^{Aa**}	1,2 ± 0,1^{aA**}
Dry season						
RS	6.8±2.1^{aB*}	9.3±3.4^{bB*}	6.7±3.9^{aB**}	7.9±2.1 ^{bB**}	2,23 ± 0,45^{Ba*}	2,7 ± 0,52^{Ab*}
U1	2.6±0.8^{aB*}	3.8±0.8^{bB*}	5.2±1.3^{aB*}	6.4±0.5 ^{bB*}	1,2 ± 0,16^{Ba*}	2,6 ± 0,0^{Ab*}
D1	1.6±0.8^{aB**}	1.8±2.8 ^{bB**}	2.2±0.3^{aB**}	3.2±1.5 ^{bB**}	1,07 ±0,11^{Ba**}	1,3 ± 0,20^{Ba**}
U2	5.7±3.2^{aB}	5.2±3.1^{bB}	4.0±2.1^{aB*}	4.5±2.3 ^{bB*}	1,3 ± 1,4^{aB}	2,0 ± 0,52^{Aa}
D2	2.5±1.7^{aB**}	3.5±2.2 ^{bB**}	3.0±0.8^{aB}	4.4±1.5^{bB}	1,2 ± 0,15^{Ba**}	1.5 ± 0,30^{Ab*}
U3	6.5±2.8^{aB*}	5.8±2.1 ^{bB*}	5.7±2.1^{aB**}	7.8±3.2 ^{bB**}	1,21 ±0,12^{Ba*}	2,5 ± 0,10^{Ab*}
D3	2.4±1.1^{aB**}	5.3±1.3^{bB**}	4.0±0.5^{aB*}	5.2±3.8 ^{bB*}	1,0 ± 0,02^{Aa**}	1,5 ± 0,4^{Ab**}

*Values represent the mean ± standard deviation. For each variable, a and b indicate significant difference between species ($p < 0.05$); A and B indicate significant difference between seasons; * and ** indicate significant differences between upstream-downstream stations and compared to the reference group (*$p < 0.001$, **$p < 0.01$).*

downstream sites (D1-D3) (F= 22.88, p<0.001) and numerically higher than the upstream sites (U1-U3) but no significant differences were observed between them (p>0.05). A significant difference was observed between seasons with higher HSI values in the rainy season (F= 5.457, p< 0.05). The K-factor of *L. pellegrini* was significantly higher than those observed in *O. niloticus* for all factors considered (F= 24.13, P< 0.001). About sex, the results observed for each of the two species showed a significant difference between males and females with higher values in females irrespective of site, season and species (F= 28.17, p< 0.001).

Gonad Histopathology

Males: Maturation stage

During the dry season, sections of *O. niloticus* testes showed all stages of spermatogenesis at all sites but not in all fish. The highest proportions in all stages were observed at the downstream sites (D1, D2, D3) except for spermatids and spermatozoa which were more represented in the testes of fish caught at the reference site (Mikyaba River) and upstream than downstream of the mining sites in the Ulindi and Mobale rivers at Mwenga and Kamituga respectively (F= 5.423 p<0.05) Sperm was found in only 5.2, 3.2 and 2.1% of the males sampled at D1, D2, and D3, respectively, compared to 45.2%, 24.6, 13.0 and 19.7% of the fish sampled at the Reference ST site (Mobale River) and upstream of the Ulindi and Mobale Rivers (U1, U2, and U3), respectively. Similar results were observed during the rainy season so all stages were rented at all sites but not in all fish. All fish at all sites released sperm, but those at contaminated sites (downstream: D1, D2, and D3) contained lower levels (F=6.181 p<0.05) of spermatids and were devoid of spermatozoa (13.4-0.0%; 9.1-0.0% and 21.7-0.0%, respectively) compared to fish from the reference site ST and those from the upstream (10.0-61.5%; 24.5-21.4%; 13.4-17.7% and 34.5-8.0%, respectively for sites (RS, U1, U2, and U3). The proportion of spermatozoa in the testis of fish from mining sites downstream of the Ulindi and Mobale rivers was significantly lower than in those from the reference site (Mikabya River) and upstream of mining sites U1, U2, and U3 (p<0.05). (**Figure-4a**).

Table 3. Seasonal and site-specific changes in Males adult *Oreochromis niloticus* and *Labeobarbus pellegrini* collected from the Ulindi and Elila river basins upstream and downstream of gold mining operations
(K: Condition Factor, RS (Reference site: Mikyaba River), Ulindi River1(U1: Upstream and D1: Downstream), Ulindi River2 (U2: Upstream and D2: Downstream), Mobale River (U3: Upstream and D3: Downstream)

Biometric indices in Males of *O. niloticus and L. pellegrini*						
	Rainy season					
Sites	GSI (%)		HSI (%)		K (%)	
	O. niloticus	*L. pellegrini*	*O. niloticus*	*L. pellegrini*	*O. niloticus*	*L. pellegrini*
RS	6.4±2.4^{Aa}	9.6±1.6^{Ab}	6.3±2.3^{Aa}	8.8±1.1^{Ab}	2,8 ± 0,6^{Aa}	1,03 ± 0,6^{Ab}
U1	2.8±1.2^{bA*}	6.1±2.4^{Aa*}	5.8±2.0^{aA}	8.4±2.9 ^{bA*}	2,3 ± 0,31^{Aa*}	1,9 ± 0,3^{Aa*}
D1	1.8±0.2^{aA**}	4.1±3,2 ^{Ab**}	3.8±1.08^{aA**}	7.5±2.4 ^{bA**}	1,69 ± 0,36^{Aa**}	1,1 ± 0,01^{Ab**}
U2	2.5±0.7^{aA*}	9.3±1.3^{bA*}	4.1±2.4^{aA*}	8.9±.3 ^{bA*}	2,53 ± 0,31^{Aa*}	1,5 ± 0,5^{Ab*}
D2	2.3±0.5^{aA**}	5.6±1.5 ^{bA**}	7.5±1.8^{aA**}	7.0±1.4^{bA*}	1,22 ± 0,30^{Aa**}	2,81 ± 0,05^{Ab**}
U3	9.0±3.0^{aA*}	11.7±2.9 ^{bA*}	3.4±1.1^{aA*}	5.4±2.8 ^{bA*}	2,0 ± 0,26^{Aa*}	1,64 ± 0,08^{Ab*}
D3	2.7±1.2^{aA**}	3.3±1.3 ^{bA**}	7.6±2.1^{bA**}	9.4±2.5 ^{bA**}	0,92 ± 0,21^{Aa**}	2,76 ± 0,05^{Ab**}
Dry season						
RS	3.5±0.3^{Ba}	9.2±1.3^{Ab}	3.6±1.4^{aB}	5.3±1.7^{bB}	2,6 ± 0,2^{Ab}	2,84 ± 0,09^{Bb}
U1	2.6±1.0^{aB*}	4.1±2.1 ^{Bb*}	4.9±3.6^{aA*}	6.9±1.3 ^{bB*}	2,6 ± 0,30^{Aa*}	2,3 ± 0,30^{Aa*}
D1	1.6±0.3^{aB**}	3.1±2.1 ^{Bb**}	2.7±1.6^{aB**}	5.0±0.9 ^{bB**}	1,26 ± 0,20^{Aa**}	2,9 ± 0,30^{Bb**}
U2	2.4±0.3^{aA*}	8.8±2.6 ^{Bb*}	3.1±0.3^{aB*}	5.0±1.8 ^{bB*}	2,2 ± 0,52^{Aa*}	2,80 ± 0,06^{Ab*}
D2	1.2±0.7^{aB**}	5.4±1.7 ^{Bb**}	4.0±1.8^{aB**}	6.4±2.1 ^{bB**}	1,8 ± 0,30^{Aa**}	2,01 ± 0,05^{Bb**}
U3	2.9±1.2^{aB*}	9.5±3.0 ^{Bb*}	2.1±1.0^{aB*}	5.8±1.1 ^{bA*}	2,3 ± 0,10^{Aa*}	1,0 ± 0,05^{Bb*}
D3	1.6±0.8^{aB**}	4.5±2.0 ^{Bb**}	6.2±2.2^{aB**}	9.3±2.0 ^{bA**}	0,65 ± 0,7^{Aa**}	2,1 ± 0,01^{Bb**}

*Values represent the mean ± standard deviation. For each variable, a and b indicate significant difference between species ($p < 0.05$); A and B indicate significant differences between seasons; * and ** indicate significant differences between upstream-downstream stations and compared to the reference group ($*p < 0.001$, $**p < 0.01$).*

Sections of fish testis collected from *L. pellegrini* during the dry season indicate the presence of all six stages of testicular development in fish caught at reference sites (RS) and upstream of mining sites (U1-U3) while those downstream contaminated sites (D1-D3) were devoid of sperm. As in the dry season, all stages of spermatogenesis were recorded in all sites during the rainy season, but not in all fish sampled and for all sites. The proportion of spermatozoa in the testes of fish at the downstream site: D1, D2, and D3 (11.4-21.3%) was significantly lower ($F= 4.23$ $p<0.05$) than during the dry season but not in all fish sampled and for all sites. The proportion of spermatozoa in the testes of fish from the downstream site: D1, D2, and D3 (11.4 to 21.3%) were significantly lower ($F= 3.602$ $p<0.05$) than in those from the reference sites RS, U1, U2, and U3 with 78.0; 25.2; 1.6 and 4.1% respectively (**Figure-4b**).

Testicular Alterations

Fish specimens sampled upstream of the mine sites and at the reference site: U1, U2, U3, and RS had the lowest ($F= 3.122$ $p<0.05$) occurrences of gonadal alterations (6-9 alterations), whereas fish sampled downstream at sites D1, D2, D3 had the highest occurrences of alterations (8-12 alterations). A total of 12 different taints were observed for both species and almost all sites in seasons **(Figure A1).** The highest degree of histological alterations was observed in the rainy season for both species together. Regressive changes (i.e. fibrosis, necrosis, vacuolation of testicular parenchyma, presence of cellular syncytium, detachment of cytoplasmic membrane, disorganization of lobular and cystic structures and infiltration of adipocytes) and intersex (presence of testicular oocytes) were found to be more prevalent across species and seasons in all sample sites, compared to any of the other response patterns**.** Except for the testicular degeneration, the presence of foam cells in the lobular lumen, and disorganization of lobular structures were present in more than 30% of testicular samples from each contaminated site **(Table A1)**. Germ cell syncytia were more frequently observed in *L. pellegrini* collected from contaminated sites than in *O. niloticus* (found in only 37.2% of tissues during the D2 flooding period). Organ alterations (Testes, Ovaries, Livers and Gills) are presented in additional data files **(Figure-5a; 5b).**

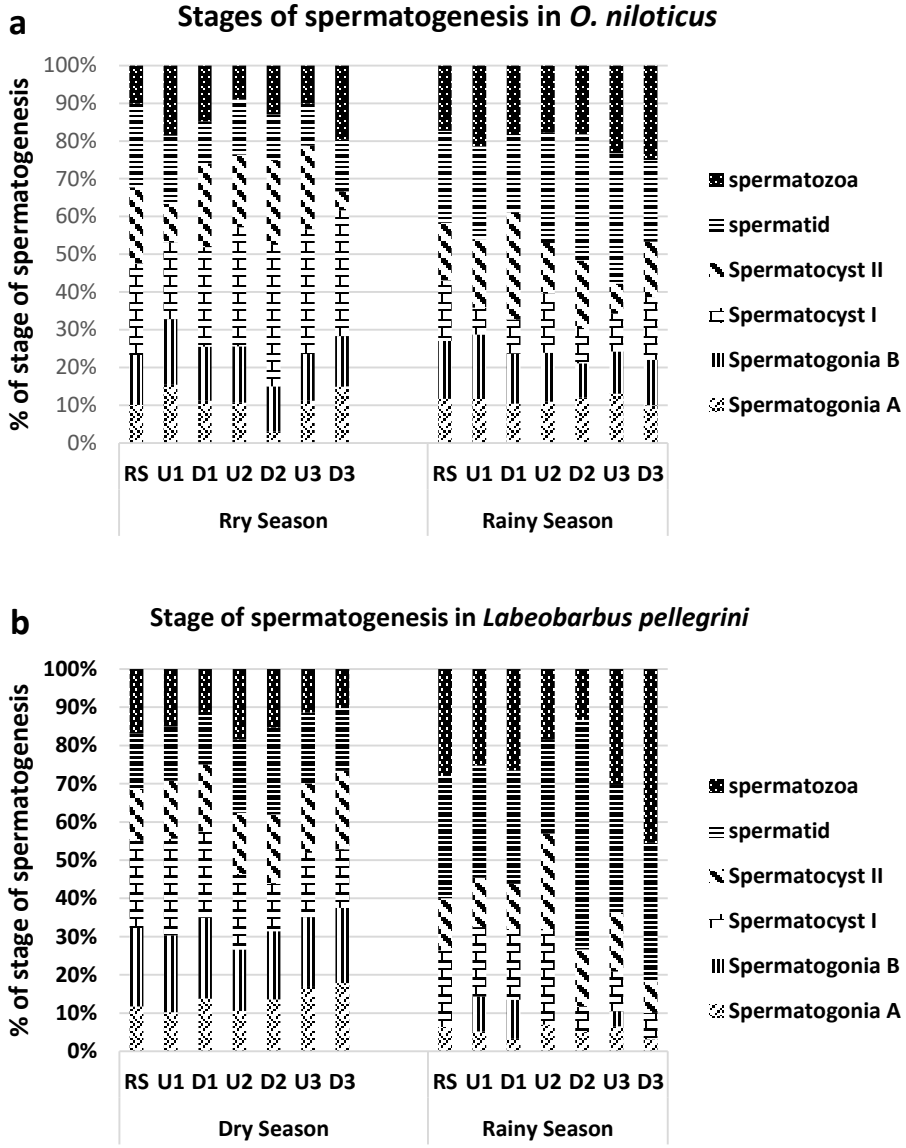

Figure-4. Percentages of different stages of spermatogenesis in
(a) *O. niloticus* ; ***(b)*** *L. pellegrini* sampled during the dry and rainy seasons at different sites upstream (U1-U2) and downstream (D1-D2) of the Ulindi, Mobale (U3: Upstream and D3: Downstream) and Mikyaba rivers (reference site RS).

Semi-quantitative analysis.

The testis alterations observed in fish sampled at the downstream sites (D1, D2, and D3) were much more pronounced than those found in fish from the upstream reference sites ($F= 6.356$ $p<0.01$). Testis alterations reached class 3 ($It \leq 35$) in *O. niloticus* sampled at the downstream mining station sites (D1, D2, and D3), compared to the upstream sites (U1, U2, and U3) where they reached class 2 ($10 \leq It \leq 25$) regardless of the season. In *L. pellegrini*, the testis alterations reached class 4 ($It > 35$) in fish from D1, D2, and D3 while class 3 was reached for fish from upstream sites (U1, U2, and U3) for all two seasons.

The presence of testicular oocytes (ovotestis) was rarely identified in fish collected upstream of mining sites, but downstream, the prevalence of ovotestis ranged from 22.5 to 75.8% in O. niloticus and from 25 to 80% in *L. pellegrini*. The highest prevalence was recorded at D2 (80%) for *L. pellegrini* and the lowest prevalence was observed at station U1 (8.3%) for *O. niloticus* ($F= 4.562$ $p<0.05$). No cases of ovotestis were observed in fish caught in the Mikyaba River considered as a reference site whereas fish collected from upstream sites had a low prevalence of ovotestis not exceeding 25% for both species, regardless of the season. In *O. niloticus*, the ovotestis observed have diameters ranging from 0.07 to 0.12 mm with an average of 0.09 ± 0.02.

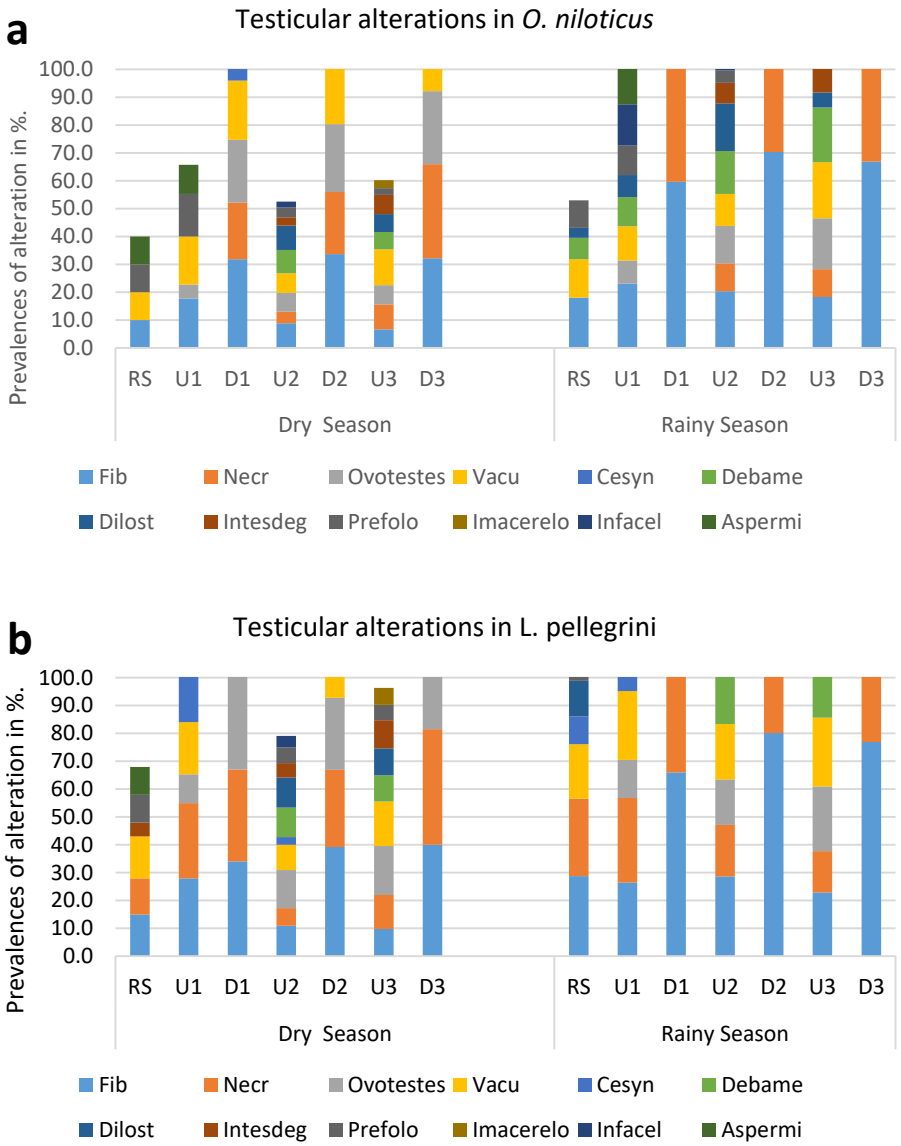

Figure 5. Testicular Alterations in (**a**) *O. niloticus* and **(b)** and *L. pellegrini* sampled during the dry and rainy seasons at different sites upstream (U1-U2) and downstream (D1-D2) of the Ulindi, Mobale (U3: Upstream and D3: Downstream) and Mikyaba rivers (reference site RS). With Fib :Fibrose, Necr. :Necrosis, Ovotestes : Testicular oocytes, Vacu :Vacuolation, Cesyn : Cellular syncytium, Debame :Detachment of basement membrane, Dilost. : Disorganization of lobular structures, Intesdeg. : Increased testicular degeneration, Prefolo : Presence of foam cells in the lobular lumen, Imacerelo : Immature cells released into the lobular lumen, infacel : Infiltration of fat cells, Aspermi : Abnormal sperm increase.

Females* : *Maturation stage

Ovarian sections of female *O. niloticus* sampled during the dry season indicated the presence of all stages of oogenesis at all sites with a marked preponderance of the perinucleolar stage (52.3-64.7%). Higher rates (F= 7.024 $p<0.05$) of oocytes in the cortical alveolar (14.5-18.2%) and early vitellogenic (8.7-14.2%) stages and lower rates (F= 2.925 $p<0.05$) of late vitellogenic oocyte stages (2.3-8.0%) were observed in fish collected from sites downstream of the mine pits compared to those upstream considered as reference sites. During the dry season, ovaries contained very few oocytes beyond the late vitellogenic stage. During floods, all stages were present in *O. niloticus* ovaries collected from all sites, with a marked preponderance of perinucleolar oocytes (40.1-74.3%). A higher proportion (F=4.301 $p<0.05$) of oocytes in the alveolar cortical (8.7-25.5%) and early vitellogenic (3.3-20.4%) stages at all sites and in all seasons. The final maturation stage was more present in the ovaries of *O. niloticus* collected from downstream sites compared to upstream mining sites. (**Figure-6a**).

In *L. pellegrini*, ovary sections from fish collected during the dry season also indicated the presence of all stages of oocyte development with a higher proportion of perinucleolar oocytes (58.9-75, 3%) and lower (F=3.312 $p<0.05$) proportions of cortical alveolar oocytes (3.5-6.3%)

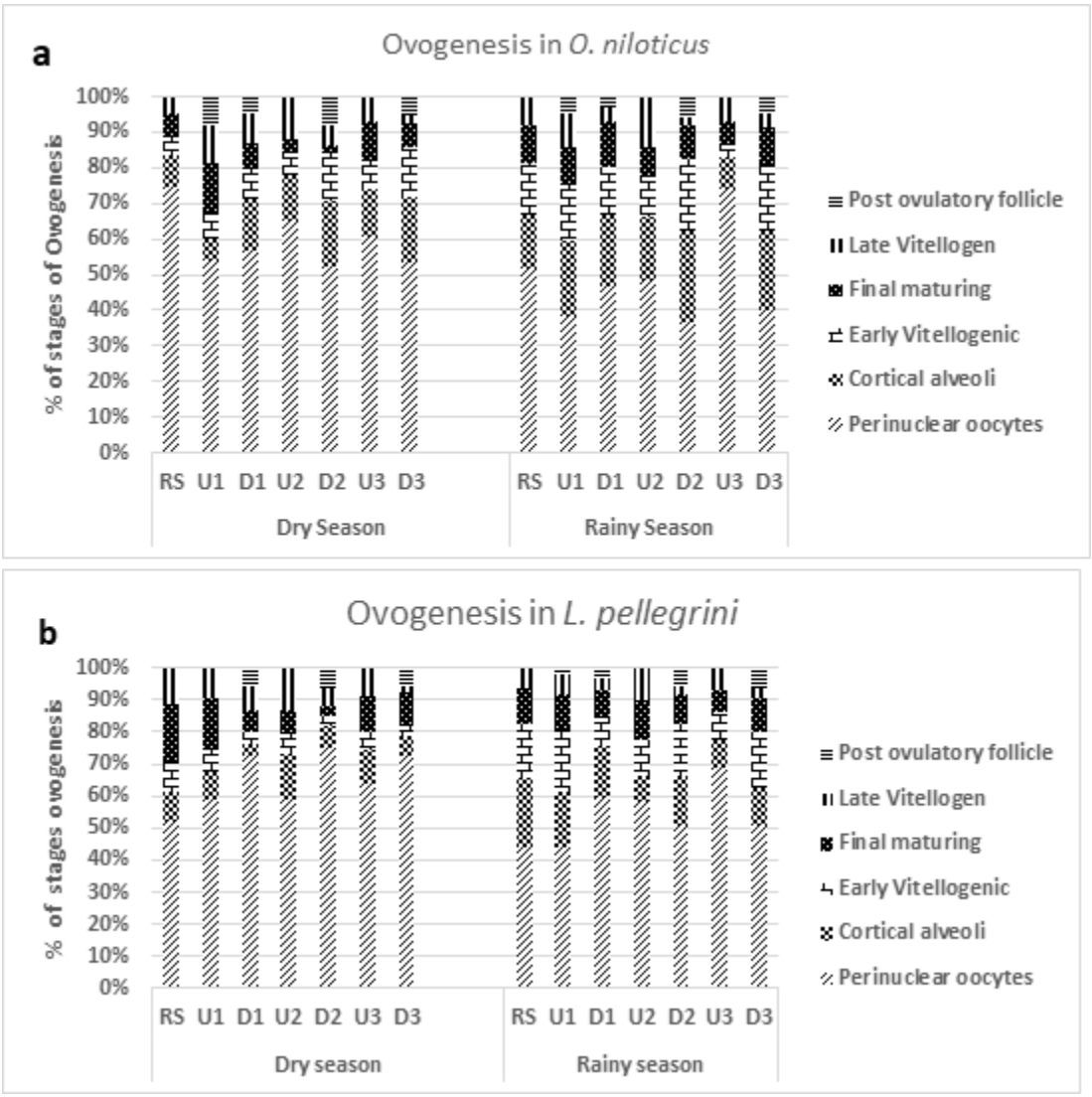

Figure 6. Percentages of different stages of ovogenesis in (a) *O. niloticus* **(b)** *L. pellegrini* with (DS: Dry Season, RS: Rainy Season, ST (Reference site Mikyaba River), Ulindi River1(U1: Upstream and D1: Downstream), Ulindi River2 (U2: Upstream and D2: Downstream), Mobale River (U3: Upstream and D3: Downstream).

and early vitellogenic oocytes (3.3-3.5%) in fish from downstream sites (D1, D2, and D3) compared to those from reference and upstream sites (U1, U2, and U3). In contrast, during the flooding period, all stages were observed in the ovaries of fish collected downstream (D1, D2, and D3), with a relatively low percentage (F=5.421 $p<0.05$) of post-ovulatory oocytes (3-6.5%) compared to those from the reference site (U1, U2, and U3) (**Figure-6b**).

Prevalence of ovarian alterations.

Six types of alterations, all classified as regressive changes (RCs) have been identified in *O. niloticus* fish and *L. pellegrini* females**.** These include necrosis, fibrose, pre-ovulatory atretic follicles, melanomacrophage centres (MMCs), vacuolation and cytoplasmic membrane detachment in perinucleolar oocytes. Regardless of species and season, fish from the reference sites (U1, U2, and U3) affiched the lowest number of alterations (0-4 damage). The most prevalent alterations in fish sampled regardless of site or season where the presence of MMC and necrosis which were observed values between (9.8-34.6%) and (9.7-56.9%) in *Oreochromis niloticus* and *Labeobarbus pellegrini* respectively during the dry season.

Semi-quantitative analysis of ovarian alterations.

The same classes of ovarian alterations (class 2 and 3) were determined at all sites, regardless of species and season, except *O. niloticus* which was sampled at the reference sites (U1, U2, and U3) during the dry season and showed less histological alterations. However, a more detailed analysis showed that the Ovarian Index (Io) of *L. pellegrini* caught at contaminated sites (D1, D2, and D3) downstream of the mine sites was closer to Class 3 than the Ovarian Index (Io) at the reference sites (U1, U2, and U3), regardless of the season. For both species, the Ovarian Index (OI) of fish from polluted sites was higher than that of the reference sites. A comparison of testis and ovarian

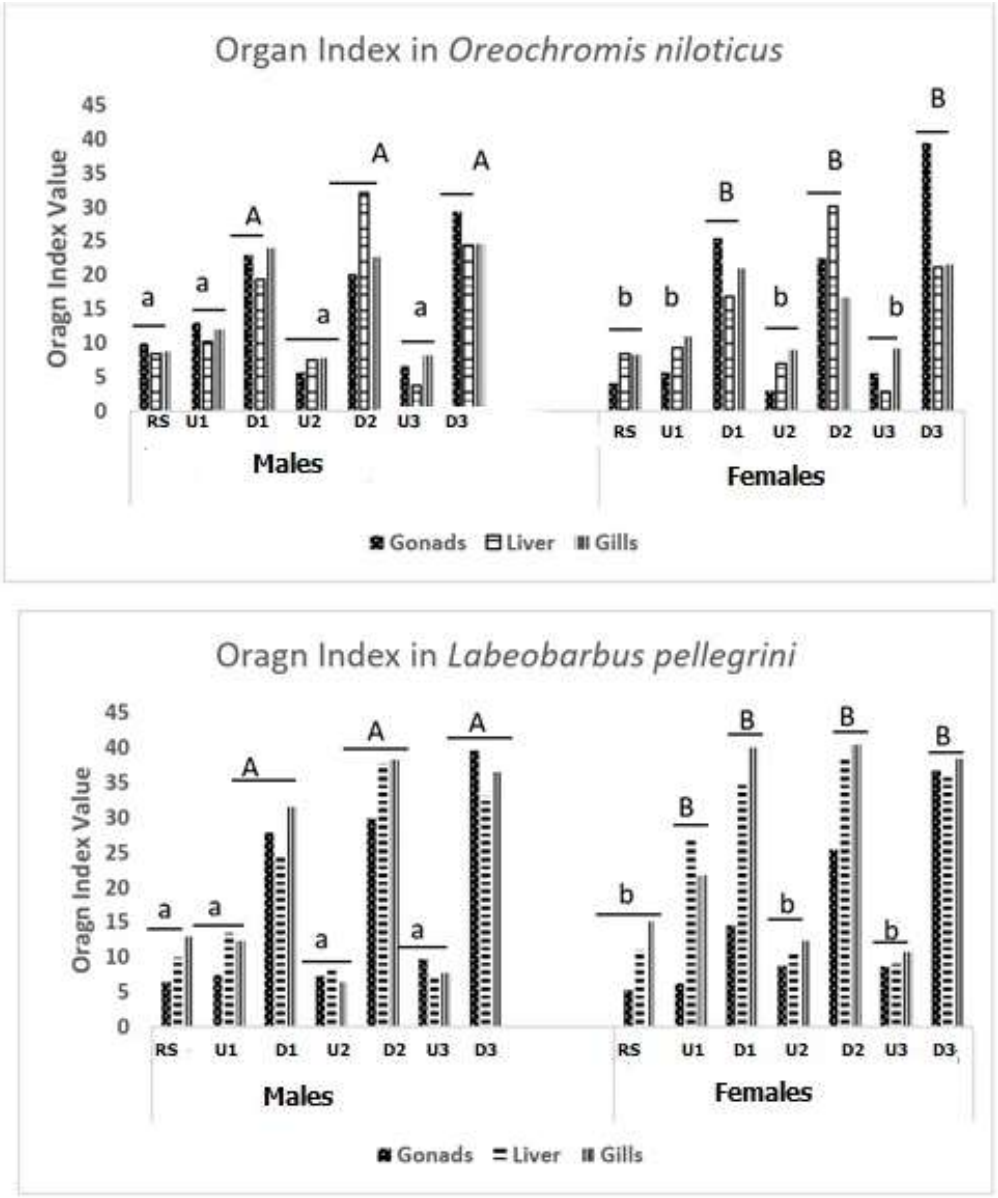

Figure-7. Male and Female organ index in *O. niloticus* and *L. pellegrini* *with* RS (Reference site Mikyaba River), Ulindi River1(U1: Upstream and D1: Downstream), Ulindi River2 (U2: Upstream and D2: Downstream), Mobale River (U3: Upstream and D3: Downstream). For each species, A and B indicate the significant difference (F= 54.61, p<0.05) between males and females and * and ** indicate the difference (F= 8.415, p<0.001) between upstream and downstream sites.

alterations in both species at each site indicated that testis structures were more affected than ovarian structures.

Liver Histopathology

Prevalence of liver alterations

In males, the highest numbers of liver alterations (8 alterations) were observed at sites D1, D2, and D3 during the rainy season in both *O. niloticus* and *L. pellegrini*. Fish collected from reference sites upstream of the mining sites showed the lowest number of liver changes (≤ 4 changes). Regressive changes were found to be more frequent in both species from all sampling sites compared to any other response pattern. MMC and glycogen depletion and macrovesicular steatosis were strongly present in more than 43.4 _ 41_ and 56.6% of liver samples, respectively, regardless of species and season. Haemorrhage, sinusoidal congestion, and fibrillary inclusion were more present in all contaminated sites (D1, D2, D3) for all sexes irrespective of species and season. In females, the highest number of hepatic alterations was observed in *L. pellegrini* from sites D1, D2, and D3 during the rainy season. Fish sampled at reference sites U1, U2 and U3 had the lowest number of alterations (≤ 6 alterations). Regressive changes were more frequent than progressive changes. MMC, simple cell necrosis, and glycogen depletion, weres present at all sites, regardless of species and season. In general, liver changes in male specimens were much more pronounced than in females. The prevalence of liver

Table-3. Total index of *Oreochromis niloticus* and *Labeobarbus pellegrini* for each site and sampling season (U1, U2, and U3: Upstream of the respective mining sites of the Ulindi, Zaly, a and Mobale Rivers; D1, D2, and D3: Downstream of the mining sites of the same rivers) and RS: Reference site, Mikyaba River.

Total Index in Male of *O. niloticus and L. pellegrini*						
	Dry Season			**Rainy Season**		
Sites	**N**	***O. niloticus***	***L. pellegrini***	**N**	***O. niloticus***	***L. pellegrini***
RS	30	$24,2 \pm 8,0^{aA}$	$24,5 \pm 10,2^{aA}$	26	$29,3 \pm 2,9^{aB}$	$27,2 \pm 7,8^{aB*}$
U1	20	$32,3 \pm 9,5^{aA*}$	$41,0 \pm 8,0^{bA*}$	25	$40,5 \pm 16,5^{aB*}$	$48,6 \pm 13,6^{aB*}$
D1	20	$66,3 \pm 10,6^{aA**}$	$84,0 \pm 11,0^{bA**}$	25	$90,5 \pm 26,5^{aB**}$	$110,6 \pm 14,2^{aB**}$
U2	19	$21,0 \pm 8,9^{aA*}$	$21,9 \pm 4,1^{aA*}$	25	$21,5 \pm 7,4^{aA*}$	$37,4 \pm 13,4^{aB*}$
D2	15	$75,0 \pm 10,6^{aA**}$	$106,1 \pm 17,8^{bA**}$	25	$113,4 \pm 23,2^{aB**}$	$111,1 \pm 23,5^{aB**}$
U3	22	$18,6 \pm 5,3^{aA*}$	$24,7 \pm 6,8^{aA*}$	25	$24,7 \pm 4,5^{aB*}$	$32,6 \pm 4,9^{aB*}$
D3	18	$78,4 \pm 9,4^{aA**}$	$109,4 \pm 20,6^{bA**}$	24	$120,4 \pm 36,9^{aB**}$	$116,0 \pm 24,7^{aB**}$

*Values represent the mean ± standard deviation. For species, a and b indicate significant difference between species ($p < 0.05$); A and B indicate significant difference between seasons; * and ** indicate significant differences between upstream-downstream stations and compared to the reference group ($*p < 0.001$, $**p < 0.01$).*

Table-4. Total index of *Oreochromis niloticus* and *Labeobarbus pellegrini* for each site and sampling season (S1, S3, and S5: Upstream of the respective mining sites of the Ulindi, Zaly, a and Mobale Rivers; S2, S4, and S6: Downstream of the mining sites of the same rivers) and ST: Reference site, Mikyaba River.

Total Index in Male of *O. niloticus* and *L. pellegrini*						
	Dry Season			**Rainy Season**		
Sites	**N**	***O. niloticus***	***L. pellegrini***	**N**	***O. niloticus***	***L. pellegrini***
RS	26	$20,9 \pm 2,7^{aA}$	$23,6 \pm 5,1^{bA}$	30	$22,0 \pm 6,5^{bB}$	$24,5 \pm 5,3^{aA}$
U1	20	$31,3 \pm 10,6^{aA}$	$34,2 \pm 7,8^{bA*}$	23	$38,8 \pm 12,6^{aB*}$	$36,3 \pm 5,4^{bB*}$
D1	20	$63,3 \pm 13,6^{aA*}$	$48,6 \pm 7,8^{bA**}$	23	$84,8 \pm 16,2^{bB**}$	$72,3 \pm 8,5^{bB**}$
U2	22	$19,0 \pm 3,1^{aA}$	$20,3 \pm 5,1^{aA*}$	23	$16,4 \pm 8,3^{aB*}$	$30,6 \pm 8,9^{bB*}$
D2	20	$69,4 \pm 31,0^{bA*}$	$87,0 \pm 23,9^{bA**}$	24	$95,1 \pm 15,1^{bB**}$	$97,7 \pm 13,8^{bB**}$
U3	19	$17,9 \pm 5,6^{aA}$	$20,3 \pm 7,1^{bA*}$	22	$22,1 \pm 0,9^{aB*}$	$30,8 \pm 6,4^{aB*}$
D3	17	$82,3 \pm 32,6^{bA*}$	$106,0 \pm 15,1^{bA**}$	29	$108,1 \pm 23,6^{bB**}$	$106,8 \pm 29,2^{bB**}$

*Values represent the mean ± standard deviation. For species, a and b indicate significant difference between species ($p < 0.05$); A and B indicate significant difference between seasons; * and ** indicate significant differences between upstream-downstream stations and compared to the reference group ($*p < 0.001$, $**p < 0.01$).*

changes was higher at the contaminated sites (D1; D2 and D3), for both species**.**

Semi-Quantitative Analysis

The liver index (Ih) of male *L. pellegreni* was higher ($F=20.7$; $p< 0.001$) at all downstream mining sites, reaching class 4 ($Ih \geq 35$) while fish from U1, U2, and U3 remained in class 2 ($10 \leq Ih \leq 25$). In general, the liver index (Ih) was higher during the rainy season than during the dry season. For *O. niloticus*, only males sampled downstream (D1, D2, and D3) during the rainy season reached class 4. As with the males, the liver index (Ih) of the female *L. pellegrini* was higher during the rainy season than during the dry season, with class 4 only being reached in D2 and D3 during the flooding, whereas the liver index of females sampled in the other from downstream mining sites never exceeded class 3. Similar observations were made for *Oreochromis niloticus*, with the liver index of females in class 2).

Gills Histopathology

Prevalence of gill alterations

Over 90% of gills observed at sites downstream of mining operations showed moderate to severe histopathological changes. The most common 'progressive' alteration is hypertrophy of the gill epithelium and fusion of secondary lamellae. Circulatory disturbances frequently occur, usually in the form of hyperemia and telangiectasia.

No significant differences ($p > 0.05$) were observed between the sexes for either species**.** The changes were less in *O. niloticus* and more intense in *L. pellegrini* ($p < 0.05$). Necrosis and lamina dimorphism were the most pronounced regressive alterations in both species at all downstream sites (D1, D2, and D3). Although epithelial lifting and lamellar folding were highest in *L. pellegrini*, lamellar disorganization was the most expressed regressive alteration in *O. niloticus*. This experiment showed that the gills of these two fish species living in the same waters react very differently to the same cocktail of pollutants, suggesting slightly different ecotoxic mechanisms between species.

Semi-Quantitative Analysis

Comparing the gill index obtained for each of the two species studied, the results in ***Figure 7***, show that the gill index (Ib) of *L. pellegreni* was higher in all contaminated sites, reaching class 4 ($Ib \geq 35$) while fish from U1, U2 and U3 did not exceed class 2 ($10 \leq Ib \leq 25$). In *O. niloticus*, only males from D3 and all females sampled at downstream sites (D1, D2, and D3) during the rainy season reached class 3 ($26 \leq Ib \leq 35$).

In general, as for the gonads and liver of these two species studied, the gill index (Ib) was higher during the rainy season than during the dry season with a highly significant difference by species, by site, and by season regardless of sex. Thus, samples of fish collected downstream (D1, D2, and D3) during the rainy season reached class 4 ($Ib \geq 35$) for *L. pellegrini* while for *O. niloticus* collected at the same locations, their gill index did not exceed class 3 ($26 \leq Ib \leq 35$).

Mercury Content in Water, Sediment, and Organs

The present study is the first in this basin to look at reproductive disturbances in target fish and also at different types of gonadal and liver alterations. Mercury was targeted because of its intensive and majority use in artisanal gold mining in the eastern DRC river catchment area, particularly in the Mwenga territory (COSOC-GL, 2015; Global Witness, 2016; Nkuba et al., 2019; Balegamire et al., 2022; Verbrugge et al., 2022).

In the studied organs, T-Hg levels were significantly higher in the testis, liver, and ovaries of fish from downstream sites compared to those from upstream ones. Several factors, such as organ-specific biological activity and metabolism, actively contribute to altering the rates of incorporation of toxic metals into different body tissues (Canuel et al., 2009). Similarly, interactions between the physical and chemical characteristics of the environment influence the retention of chemical pollutants by biological organisms (Borgå et al., 2004; Canuel et al., 2009; Soler et al., 2020). The practice of artisanal gold mining, where mercury is used to amalgamate the gold after washing the mined soil in the Ulindi and Elila river basins where the study is conducted, has led to the release of mercury into the river. Like quicksilver, mercury occurs in nature in various forms, as it is released into the environment through natural processes, such as volcanic eruptions, rock weathering and forest fires, and a number of anthropogenic activities (UNEP, 2002). Mercury does not break down in the environment, but rather is recycled between the atmosphere, land and water, and can travel great distances from its original source (Gabriel & Williamson, 2004). Mercury is a toxic metal capable of bioaccumulation and biomagnification in the food chain and can cause numerous health risks to humans who consume it directly or indirectly. Mercury exposure has been confirmed to cause diseases such as cancer, neurological disorders, autism, gastrointestinal disorders, respiratory tract irritation, kidney failure, disrupted immune processes and, in some cases, death (Oladipo et al., 2013).

Mercury being a neurotoxin, its form (methylmercury or elemental mercury), its amount in the exposure, the age of the exposed person, the duration of the exposure, the way the person is exposed (breathing, eating, skin contact,...) as well as the health of the exposed person are among the factors that affect the health of a person exposed to mercury (Hyman & Kohl, 2004; F. J. Risher & Amler, 2005; Wojcik et al., 2006; Brodkin et al., 2007; Rafati-Rahimzadeh et al., 2014; US EPA, 2015a; Magos & Clarkson, 2016).

Studies conducted in humans, monkeys, and rodents have shown that gastrointestinal absorption of mercury is close to 100% following ingestion of methylmercury chloride or when incorporated into fish or other ingested protein. Dimethylmercury is rapidly absorbed through human skin, while acute inhalation of metallic or inorganic mercury vapours is mainly responsible for pulmonary damage, while chronic inhalation is responsible for neurological (encephalopathy) or renal (tubulopathy, glomerulonephritis) damage (Mergler & Lebel, 2001; Goullé & Guerbet, 2016; Goldgewicht, 2002; Bose-O'Reilly et al., 2008; Bensefa-Colas et al., 2011). The effects of mercury on wildlife can include mortality (death), reduced fertility, slower growth and development, and abnormal behaviour that affects survival, depending on the level of exposure (US EPA, 2015a). In addition, research indicates that the fish endocrine system, which plays an important role in fish development and reproduction, may be altered by environmental levels of methylmercury (B. Mandiki et al., 2014; US EPA, 2015a).

Mercury enters the air from a number of sources and is eventually deposited in water bodies such as lakes and rivers, or on land, where it can be washed into the water. Microorganisms in water bodies can convert it to methylmercury, which then accumulates in fish and shellfish (US EPA, 2015b; Johnson-Arbor et al., 2021). Methylmercury levels in fish and crustaceans depend on what they eat, how long they live and their position in the food chain (Anani & Olomukoro, 2019; Risher et al., 1999). In a given water body, the highest concentrations of

methylmercury are generally found in large fish that eat other fish. According to US EPA, (2015), almost all methylmercury exposures in the US occur through the consumption of fish and shellfish that contain high levels of methylmercury.

There was also a seasonal difference in T-Hg content in all organs with the highest values in the rainy season except for the ovary where high mercury concentrations were observed in the dry season for both species. The high level of mercury in these different organs during the rainy season could be due to its high accumulation in the aquatic environment, especially in the sediments during this period. Furthermore, significant seasonal changes in ovarian tissue may be due to differences in water quality, gonadal maturity ty, and environmental conditions (Abdel-Khalek, 2015; Heba & Mohamed, 2019). According to Thomassin et al., (2003) the trend of the metal to bioaccumulate in the gonads during the dry season could be explained by its ability to bind to proteins transferred for gamete metabolism. On the other hand, the high values of heavy metals during the rainy season could be attributed to runoff discharges (Goussanou et al., 2018; Kouamenan et al., 2020). Wastewater and runoff water loaded with mining and domestic waste from detergents, bacteria, cosmetics and other hygiene products used by the surrounding populations are the potential sources of toxic metal contamination of the Ulindi River in Mwenga and Mobale in Kamituga.

The result of the chemical analysis carried out on water and sediment in the present study was between 0.6-0.96 mg. L^{-1} and 0.04-0.7 mg. L^{-1} respectively. The average concentration of mercury in sediment is 0.212 mg.kg-1 and 0.74 mg. L^{-1} in water. This value is higher than the limit of 0.105 $mg.kg^{-1}$ for sediment and 0.01 mg. L^{-1} for water set by USEPA and WHO/FAO respectively. WHO, 2003 reported that, even any area without Hg emitting source, can be enriched by Hg because of global Hg cycling occurs through the air and water.

In fish, the concentration of mercury in all organs analysed ranged from 0.06 - 2.13 $mg.kg^{-1}$ and 0.09 - 3.81 $mg.kg^{-1}$ in *O. niloticus* and *L. pellegrini* respectively. These values were higher than the regulatory limit of 0.0001 ppm for its presence in environmental samples. This is thought to be due to the rapid growth rate of *L. pellegrini*, its trophic position and high metabolism which can lead to higher mercury concentrations over short periods of time as in the case of the wahoo, *Acanthocybium solandri*, from the waters off the southeastern United States and the Bahamas (Adams, 2010).

These results show that *L. pellegrini* accumulated more T-Hg than *O. niloticus*. The T-Hg level was higher in the organs of both species during the rainy season than during the dry season. Furthermore, the T-Hg level varied significantly between the organs tested in the following order: Gonads > Liver > Gills. The level of T-Hg exceeded the WHO/FAO recommended limit (T-Hg = 1.0 mg/kg wet weight) only in the testes and gills of *L. pellegrini* collected from the downstream sites (D2 and D3) of the Ulindi and Elila rivers, but this does not necessarily reflect the level in the carcass.

Analysis of the results of this study also showed that the organs of *L. pellegrini* are more contaminated with mercury than those of *O. niloticus* from the same sites. The heavy metal contamination of the organs of this fish species is thought to be related to its feeding behaviour. According to Mukabo et al (Under review), the food spectrum of *L. pellegrini* consists of fish, insects, arachnids, and debris. This species has an omnivorous diet with a piscivorous tendency. Therefore, the position of this species in the food chain could justify the high contamination of its organs by metallic elements.

Histopathology of the Gonads

For both species studied, all germ cell stages involved in gametogenesis were observed in males and females. However, in males, structural abnormalities of testicular tissue were observed. Indeed, the effects of pollutants on the testis of fish are widely documented concerning the disturbances gonads. In the current study, the structure of the seminiferous tubules, fibrosis and necrosis were the most observed alterations in both sexes for *O. niloticus* and *L. pellegrini,* whereas in males, a predominance of testicular oocytes (highest prevalence rate in *L. pellegrini*) was observed in both species during the rainy season. This could be explained by the high bioaccumulation of pollutants in these organs during this period when mercury accumulates much more in sediments downstream of mining sites in the Ulindi and Elila rivers.

Regarding the occurrence of histological alterations by sex and site, the results of this study show that females are less affected than males for all species. Also, a high number of alterations were observed during the dry season, but a high prevalence of each of these alterations was found during the rainy season for fish collected in the downstream sites (D1-D3). This is due to the fact that mining activities in the study area are intensified during the dry season. The high prevalence of tainting in fish caught downstream during the rainy season is justified by the increased concentration of mercury in water and sediment from runoff from surrounding land, as it is at the river's edge that cleaning and amalgamation of fine gold particles take place. The most common alteration at the upstream sites (U1-U3) was the presence of melano-macrophagic centre (MMC). MMCs act as focal deposits for resistant intracellular bacteria, from which chronic infections can develop (Knoth & Zdrahal, 2013). Upstream, these alterations were most likely due, on the one hand, to an evasive migration of already debilitated fish downstream to upstream, and on the other hand, these upstream alterations are related to the low levels of T-Hg detected in the upstream sites. However, an increase

in the prevalence of SMC at downstream sites (D1-D3) may indicate oocyte degeneration (Roberts, 2001).

Comparing the ovaries of *L. pellegrini* and *O. niloticus*, the results of this study show that the females of the former species (*L. pellegrini*) were more frequently altered than those of *O. niloticus*, probably due to their trophic position and benthic preferences, putting them in close contact with sediment pollutants. There are also very different behaviour and reproductive strategies of each species. Of the high-importance alterations, factor (3), pre-ovulatory atretic follicles, were the most prevalent in both species and had the highest percentages during the rainy season at the polluted downstream sites (D1-D3). Pre-ovulatory atretic follicles have been identified by several authors as key histological features indicating chemical-induced endocrine disruption (Van den Belt et al., 2002; Soler et al., 2020; Ghosh et al., 2022) and influence more the variation of the transcriptional profile (Villeneuve et al., 2010). Several authors classify heavy metals as endocrine disruptors (EDs). The latter (EDs) are synthetic or natural chemical molecules present in the environment that have the potential to negatively affect the homeostasis of the endocrine axis leading to neurological, developmental, immunological and reproductive disorders in the body (Chen et al., 2020; Djemaouni et al., 2020).

Heavy metals are important aquatic pollutants that can interfere with physiological and biochemical processes in aquatic organisms including the endocrine system. For example, the study by Cao et al., 2019 found toxic effects of copper (Cu) on the endocrine system of zebrafish (Daniorerio) exposed in vivo to 0, 10, 20, 40 $\mu g.L^{-1}$ for 30 days. These effects are reflected in the altered expression of male genes in the gonads and brain, in the disruption of E2, T and 11-KT levels and in the reduction of body weight and gonadal development of copper-exposed fish compared to the control. On the other hand, the study by Chen et al., 2020 indicates that cadmium exhibits estrogenic effects in juvenile male tilapia (*Oreochromis niloticus*) manifested by an increase in the relative expression ratio of vitellogenin (vtg2) mRNA after exposure to 2.865 µM for 7 days. Huang et al., 2020 detected heavy metals (Cr, Mn, Ni, Cu, Zn, Cd and Pb) and a metalloid (As) with maximum concentrations that can reach up to 2.67, 77.4, 0.63, 2.56, 30.5, 0.11, 2.56, 2.76 $\mu g.L^{-1}$, respectively.

Several studies done on water and sediment matrices in different aquatic ecosystems around the world show that water and sediment, which are the main pathways of contamination for fish, are good receptors and important stocks for the accumulation of endocrine disruptors (Roméo et al., 1999; Fernandes et al., 2008; Sarkar et al., 2016; Qin & Tao, 2022; El-Maksoud et al., 2022) . On the other hand, the body of research carried out on aquatic organisms, in particular on fish exposed to well-known concentrations of chemicals, shows that these substances have the capacity to interfere with the endocrine system, even at low concentrations (Abdel-Baki et al., 2011; Torres et al., 2016; Rajeshkumar & Li, 2018a; Hossain, 2020; Wickrama-Arachchige et al., 2022).

Furthermore, variation in the different parameters was dependent on the different seasons at the different sites, particularly at the reference site, indicating that seasonality of reproduction is evident for both species in the GSI and the stages of spermatogenesis and ovogenesis found in the testes and ovaries, respectively.

A relationship between heavy metals and the presence of testicular tubule damage has been reported in other studies (Pieterse, 2004; Unal et al., 2007; Nuhu et al., 2014; Zeng et al., 2020; Allouko et al., 2021; Aydın & Tunca, 2022). Testicular cell damage can affect reproductive performance by disrupting sex hormone production and producing poor-quality sperm (Çek & Yilmaz, 2007; Van Dyk & Pieterse, 2008; Marchand, van Dyk, et al., 2009; Fersaoui et al., 2018; Kouassi et al., 2018; Zaghloul et al., 2020). As in the testis, several alterations have been observed in ovarian tissue. Among the main factor alterations, atretic follicles, fibrosis, and necrosis were prevalent in gonadal tissues. According to Ambani, (2015) atresia may be due to environmental stress. It is an endocrine disruption anomaly induced by chemicals of polluting nature (Louiz et al., 2018; Reyene & Nadia, 2022). However, follicular atresia can be associated with other factors, including overpopulation (Levavi-Sivan et al., 2004; Unal et al., 2007), lack of males (Trippel & Harvey, 1990) or nutritional deficiencies (Cardona et al., 2019; Simó-Mirabet et al., 2018; Sadoul et al., 2020; Higuchi et al., 2021). Atresia is an irreversible degenerative process by which the oocyte loses its integrity and is eliminated before ovulation (Kouamenan et al., 2020). Therefore, the atretic follicle or testicular oocyte may be one of the causes of reduced reproductive success. The effect of pollutants on reproductive success is supported by some authors (Roex et al., 2001; Daouk et al., 2011).

Histological effects similar to those found in the present study have been described by authors in fish exposed to doses of insecticides or heavy metals (Kravitz et al., 2000; Marty et al., 2003; Milla et al., 2011; Agbohessi et al., 2015; Louiz et al., 2018; Abiya et al., 2018; Funmilayo et al., 2019; de Vaufleury & Gimbert, 2021a, 2021b; Maiga et al., 2022; Roosta et al., 2022; Toko et al., 2018). Alterations in ovarian follicles and spermatogenic cells may reflect damage to the gametogenesis process and affect the number of gametes produced, which may reduce the reproductive capacity of individuals in the long term (Arellano-Aguilar & Constantino Macías Garcia, 2008).

Histopathology of the Liver

The liver plays a crucial role in detoxification and biotransformation processes. Furthermore, due to its function, position, and blood supply, the liver is one of the organs most affected by the presence of water pollutants (Roberts, 2001; Van der Oost et al., 2003; Genten et al., 2010), and it has been used as a reference for the analysis of tissue damage caused by environmental toxic compounds (Amaral et al., 2002). The hepatosomatic index (HSI) is the first macroscopic parameter that reflects the condition of the liver. In this study; in contrast to the GSI, the HSI decreased significantly from upstream (U1-U3) to downstream (D1-D3)) for both species . This increase was most notable at D3. There was also a significant difference between sites D1-D3.

The results obtained in this study with regard to the Somatic Hepato Index, show that HSI is not a good indicator of pollution as the Gonado-Somatic Index and the Fulton K factor. Our results corroborate with those of Fernandes et al., 2008; Adeogun et al., 2016; Chen et al., 2020; Shaimaa et al., 2022; Taslima et al., 2022.

Liver tissues were also subjected to qualitative and semi-quantitative histological analysis for each of the two species studied. The detoxification function of the liver makes it particularly exposed to contaminants (Bazzi & Djoudad-Kadji, 2018; Fersaoui et al., 2018; Oso & Odaiba, 2022). This justifies its use as a reference for the analysis of damage caused by chemical compounds (Amaral et al., 2002). Various liver lesions were examined 8 types of alterations were identified in males and females of *L. pellegrini* and O. *niloticus*. These lesions had mainly a regressive character observed in fish from contaminated sites (D1-D3). Progressive and circulatory lesions were less represented for both species studied, in particular in males of both species, haemorrhage was the most observed circulatory disorder with a high prevalence at site D2 while sinusoidal congestion was the circulatory disorder that was more observed in the livers of females from contaminated sites during the rainy season. The high level of mercury in the livers of these two species studied could explain these results. Indeed, histological changes in the liver associated with Hg exposure or other pollutants have been sufficiently documented in many studies (Veiga et al., 2005; Schwindt et al., 2008; Marchand, Van Dyk, et al., 2009; Vergilio et al., 2012; Van Dyk et al., 2012; Maceda-Veiga et al., 2013; Kristensen et al., 2014; Jabeen et al., 2018; Lukhwareni & van Dyk, 2018; Briaudeau, 2019).

Most of the alterations identified in the liver are reversible. However, the persistence of stressors disrupts the function of nutrient synthesis while a correlation has been established between hepato-somatic and gonado-somatic relationships. This correlation reflects the use of liver reserves during gonadal maturation ((Benchikh et al., 2018; Rachida & Hadjer, 2018; Zeyneb & Mohdeb, 2018; Baali et al., 2021).

Histopathology of Gills

The results obtained from the histopathological analysis of fish gills from the Ulindi and Elila River suggest that, during the sampling period, the water and sediment contained pollutants that could induce the histopathological alterations observed. Although the observed alterations are not pollutant specific, they could be related to previously published results of gill alterations caused by experimental exposure to certain pollutants. Concentrations of mercury close to the values observed in the Ulindi River under experimental conditions induced gill lesions in the form of hypertrophy, epithelial hyperplasia, fusions, epithelial uplift and rupture, haemorrhages, and necrosis (Z. Wu et al., 2012; Aly, 2016; Lujić et al., 2015). Epithelial lifting and swelling, hyperplasia with lamellar fusion, hyperemia, telangiectasia, and chloroid cell alterations were common lesions in the gills of fish experimentally exposed to copper (Liu et al., 2010; Barišić et al., 2015) Lujić et al., (2014) observed chlorine-induced epithelial lifting, lamellar fusion, and telangiectasia. Differences in reaction patterns between species may be induced by various environmental conditions and species-specific responses to pollution(Marselli, 2020).

More than 90% of the gills observed at sites downstream of mining operations showed moderate to severe histopathological changes. No significant differences were observed between the sexes for either species. This would be due to the fact that the gill is the primary target organ for exposure in fish and is an important site for heavy metal entry (Patnaik et al., 2011; Rajeshkumar & Li, 2018a). The changes were less significant in *O. niloticus* and more intense in L. pellegrini. This experiment showed that the gills of these two fish species living in the same waters respond very differently to the same pollutant cocktail, suggesting slightly different ecotoxic mechanisms between the species. In general, as for the gonads and liver of these two species studied, the gill index (Ib) was higher during the rainy season than during the dry season with a highly significant difference by species, by site and by season regardless of sex. This is believed to be due to the high concentration of total mercury in water and sediments during the rainy season. The level of T-Hg was higher in the organs of both species during the rainy season than during the dry season. According to Rajeshkumar & Li, 2018 ,the seasonal variation of metals in fish species could be due to physicochemical and biotic factors in the environment, which influence the bioavailability of metals. The concentration of total mercury in the edible parts of two fish species studied was not assessed in this study. However, the level of T-Hg varied significantly between the organs tested in the following order: Gonads > Liver > Gills. and even though fish liver, gonads and gills are rarely consumed, this may represent a good biomonitor of metals present in the surrounding environment. Several studies have shown that the heavy metal levels in fish also vary between

species and different aquatic environments and that their bioaccumulation in fish organs is significantly correlated with fish species (Abdel-Baki et al., 2011; Adams, 2010; Ahmadi et al., 2022; Boudou et al., 1980; Hossain, 2020; Rajeshkumar & Li, 2018a; Roméo et al., 1999; Sheikhzadeh & Hamidian, 2021; Tamele & Vázquez, 2020; Torres et al., 2016; Zhang et al., 2020). The results observed in this study corroborate those obtained by these authors.

The level of T-Hg exceeded the WHO/FAO recommended limit (T-Hg = 1.0 mg/kg wet weight) only in testes and gills of *L. pellegrini* collected from the downstream sites of the Ulindi and Elila rivers. The affinity for metal uptake in contaminated water and food may differ depending on ecological requirements, metabolism and contamination gradients in water, food and sediment, as well as other factors such as salinity, temperature and interacting agents (Rajeshkumar & Li, 2018b).

According to (Lujić et al., 2014), epithelial hyperplasia and uplift are the two main defence mechanisms of fish gills when exposed to xenobiotics. Epithelial hyperplasia (progressive alteration) reduces the respiratory surface area, while epithelial uplift (regressive alteration) increases the water-blood barrier. The differences in gill responses observed in the present study may be due to the ecology and bioenergetics of these two fish species. *L. pellegrini* is an omnivorous fish and a benthopelagic inhabitant, mostly active and predatory with intense migratory movements during spawning (Lévêque & Daget, 1984; Winfield & Nelson, 2013; Eyayu & Getahun, 2022) whereas the Nile Tilapia, *O. niloticus* is mainly herbivorous living in the water column (Britton et al., 2009; Alam et al., 2015; Khallaf et al., 2018). It could be assumed that *O. niloticus* has a lower metabolic rate than the fast-swimming *L. pellegrini*, thus consuming less oxygen during regular activities. In turn, this may allow *O. niloticus* to develop a defence mechanism in the form of epithelial lifting. (regressive alteration) that increases the water-blood barrier, affecting oxygen uptake much less.
Most studies report that fish gills respond in a non-specific manner, implying that various pollutants can cause similar reactions and alterations of the gill tissue, and thus, these gill alterations can be considered the result of a generalized stress response (Poleksić & Mitrović-Tutundžić, 1994; Lujić et al., 2014; Barišić et al., 2015; Azadbakht et al., 2019b; Hodgson et al., 2020). However, the present study indicates that it is possible to detect some differences in gill response to pollution between species. Although the gills of all fish species analyzed showed progressive, regressive, and circulatory alterations, there was a significant difference in the level of expression of these response patterns. While *Labeobarbus pellegrini* showed significantly higher progressive alterations, mainly epithelial lifting, *O. niloticus* showed significantly higher regressive alterations, mainly lamina folding.

Health Indicators

The quantitative indicators of fish health used in this study (condition factor) have been widely used and discussed in the literature and were selected to reflect the overall health of the fish body. In general, the weight and condition factor K of fish collected from upstream sites (U1-U3) were lower than those from downstream (D1-D3). Several authors had already reported the decrease of condition factor K in fish (Sciaenops ocellatus, *O. niloticus*, *C. gariepinus*, and *zebrafish*) exposed to pollutants (Alvarez & Fuiman, 2005; Cook et al., 2005; Hanson et al., 2007). Several hypotheses could explain the decrease in condition factor, but the most influential one is most likely bioenergetics. The presence of environmental pollutants can affect the consumption and absorption of food by fish (Marchand et al., 2008). According to Abarshi et al. (2017), Abnormally high concentrations of metals can be toxic to fish and other aquatic organisms directly or, by extension, to humans who frequently consume these contaminated fish. Even if fish are properly fed, a significant portion of the nutrients from feed catabolism could be converted to energy to meet the energy demand caused by xenobiotic-induced chemical stress (Arunachalam et al., 1980).

During the rainy season, the testicular index of *O. niloticus* at the Downstream site (D1-D3) varied between 26 and 35 for D1 and D2 while for D3 it was even higher than 35; in L. pellegrini during the same rainy season, the testicular index was higher in all contaminated sites (D1-D3); these values are considered by (Van der Oost et al., 2003; Van Dyk & Pieterse, 2008; Van Dyk et al., 2009; N'guessan et al., 2021) indicators of deep testicular alterations with severe damage. (Van Dyk et al., 2012)and (Agbohessi et al., 2015) also found a similar liver index in African catfish sampled in the polluted Roodeplaat River in South Africa and the Alibori River in Benin respectively.

During the dry season, testes, liver, and gills in both species also showed pronounced alterations at the downstream sites (D1-D3). A comparison between seasons indicated that the alterations of these three organs increased during the rainy season. *L. pellegrini* suffered more damage than *O. niloticus*. This may be explained, to some extent, by its trophic position and benthic feeding habits and, as a corollary, its different bioenergetic capacity to that of *O. niloticus*. However, although fish from contaminated sites showed severe damage to the ovaries, alterations in the female gonads were generally less than those observed in the testes and were more pronounced during the wet season than during the dry season. Regardless of species, sex and season, the gills were more altered than the liver and gonads.

Based on the histology of the liver, gonads and gills, the total index above 35 in L. pellegrini from the downstream sites suggests that the organs examined were severely damaged, in contrast to the upstream sites where slight to moderate alterations were observed. Paradoxically,

relatively high levels of mercury were observed in the gonads of fish from some of the upstream sites, although less represented than those from the downstream sites. The presence of mercury in the upstream sites is thought to be due to an evasive migration of already debilitated fish downstream to the upstream sites, or the fact that these upstream alterations are related to the low levels of T-Hg detected in the upstream sites, see figures. These data suggest a combined effect of several chemical parameters in causing severe tissue damage in the gonads of downstream sites. The effect of the impact of artisanal gold mining seems to be visible downstream of the mining sites and is reflected in the deterioration of the general health of the fish which is caused by the concentration of mercury and the time of exposure which determines the severity of the damage. Several authors have shown that ovarian follicles, spermatogenic cells and liver tissue reflected damage or gametogenesis processes and is likely to reduce the reproductive capacity of individuals in the long term (Harianja et al., 2020; Kolie et al., 2019; Marchand, van Dyk, et al., 2009; N'guessan et al., 2010; Stoffersen et al., 2018; Weleabzgi et al., 2021; M. Wu et al., 2021).

The qualitative or quantitative indicator of fish health used in this study has been widely discussed in the literature and its selection reflects the overall organisational health of fish (Luthra et al, 2017; Van Dyk et al., 2009, 2012; Agbohessi et al., 2015; Naïja et al., 2018; Kouamenan et al., 2020; Douny et al., 2021; Beghin et al., 2021; Roosta et al., 2022). Considering the histological results, the response between the two sexes in both species suggests that the testes appeared more sensitive to chemical contamination such as mercury than the ovary. These results corroborate those reported by Ibtissem et al. (2009) in the Bizerta lagoon, Tunisia, and are contrary to those found by Kouamenan et al., 2020 in two Cichlid species (*Hemichromis fasciatus* and *Tilapia zillii* × *Tilapia guineensis*) caught in the Ebrié lagoon, Côte d'Ivoire.

Conclusion

Fish histology was used as a tool to monitor the health status in the Ulindi and Elila river catchments where mercury use is continuous, using qualitative and quantitative assessment methods. The results showed the existence of various alterations in the tissues of the organs studied in the downstream and upstream areas. Furthermore, this work suggests that mercury exposure, a pollutant with reproductive disruption capacity, has a spatial distribution that supports the usefulness of *L. pellegrini* as a sentinel fish model in the Ulindi and Elila rivers. It can also be concluded that, despite the alterations identified in the histological assessment, the organs of the fish in these two rivers appear to be in a functional state, and therefore the health of the fish in this population does not appear to be seriously compromised. But to prevent any health risk, we suggest an analysis of all chemical pollutants that may affect the health of fish in the Ulindi and Elila river basins in the east of the Democratic Republic of Congo.

Declaration of competing interest and ethical responsibilities of authors

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors

Authorship Contribution Statement

Mukabo Okito Gabriel involved in all phases of the work from conceptualization, methodology; investigation; data analysis, writing –original draft; writing – review editing and visualization. Prudencio Tacgégnon Agbohessi involved in methodology; review and editing. Syaghalirwa Mandiki Robert supervised all laboratory work and participated in the methodological development, revision and editing. Mulongaibalu Mbalassa involved in methodology; review and editing. Joseph Masirika Matunguru participated in investigation, review and editing. Jean-Claude Micha involved in conceptualization; methodology; review et editing. Gaspard Ntakimazi is the local administrator of the project and hes been involved in conceptualization; methodology; review and editing. Venant Muderhwa Nshombo involved in conceptualization, methodology, review and Editing. Thierry Jauniaux involved in Methodology and Review. Patrick Kestemont involved in the administration of the project and the acquisition of funding for this study.

Statement on the Availability of Data and Materials

The authors declare that the data and materials for this study are indeed available and can be submitted upon request.

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Compliance with ethical standards

This study was an observational and experimental study . All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institutions at which the studies were conducted.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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