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Microbiological water quality and gill histopathology of fish from fish farming in Itapecuru-Mirim County, Maranhão State

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ABSTRACT. The present study evaluated the microbiological water quality and tissue lesions in gills from Nile tilapia (*Oreochromis niloticus*) and hybrid tambacu (*Colossoma macropomum* female x *Piaractus mesopotamicus* male). For this, water and gills were collected from fish farming at six locations in Itapecuru-Mirim County, Maranhão State. Microbiological water analyses revealed contamination by total coliforms, *Escherichia coli* and heterotrophic bacteria. In the gills, we observed a diversity of Gram-positive and Gram-negative bacteria. The tissue lesions were: lamellar fusion, interlamellar hyperplasia, sub-epithelial edema and telangiectasia. Inflammatory lesions were not observed. Significant statistical difference ($p > 0.05$) was not detected when comparing different gills lesions during rainy and dry season. The correlation between lesion and pond type was statistically different ($p < 0.05$) for lamellar fusion and interlamellar hyperplasia which occurred more frequently at ground ponds. Regarding the frequency of lesions in the different fish species, there was statistical difference ($p < 0.05$), and the tambacu was more sensitive to lamellar fusion while tilapia was more sensitive for the other lesions. In relation to the sampling stations, there was statistical difference for all the gill lesions. In conclusion, tissue lesions are nonspecific and function as a defense mechanism against polluted aquatic environments, without infectious character.

Keywords: coliforms, water, lesions, gill epithelium, tilapia, tambacu.

Qualidade microbiológica da água e histopatologia de brânquias de peixes provenientes de pisciculturas do município de Itapecuru-Mirim, Estado do Maranhão

RESUMO. Com o objetivo de avaliar a qualidade microbiológica da água e as alterações teciduais em brânquias de tilápias do Nilo (*Oreochromis niloticus*) e do híbrido tambacu (*Colossoma macropomum* fêmea x *Piaractus mesopotamicus* macho), coletou-se água de pisciculturas e brânquias de peixes de seis localidades do município de Itapecuru-Mirim, Estado do Maranhão. O exame microbiológico da água revelou poluição por coliformes totais, *Escherichia coli* e bactérias heterotróficas. Nas brânquias, observou-se uma variedade de bactérias Gram-positivas e negativas. As alterações teciduais observadas foram fusão de lamelas, hiperplasia interlamelar, edema subepitelial e telangiectasia, não sendo observadas lesões inflamatórias. Não houve diferença estatística ($p > 0,05$) quando se comparou diferentes tipos de lesões branquiais com os períodos de chuva ou de seca. A correlação de lesões e tipos de tanque demonstrou diferença estatística ($p < 0,05$) para fusão de lamelas e hiperplasia interlamelar que ocorreram com maior frequência em viveiro de terra. Quanto à frequência de lesões nas diferentes espécies de peixe, houve diferença estatística ($p < 0,05$), sendo o tambacu mais sensível para fusão de lamelas, enquanto que na tilápia as demais lesões foram mais frequentes. Em relação aos pontos de coleta, houve diferença estatística para todas as alterações branquiais. As alterações teciduais encontradas são inespecíficas e agem como mecanismo de defesa frente ao ambiente aquático poluído, mas sem demonstrar caráter infeccioso.

Palavras-chave: coliformes, água, lesões, epitélio branquial, tilápia, tambacu.

Introduction

Environments rich in organic matter favor the proliferation of microorganism (FRERICHS, 1989; WALTERS; PLUMB, 1980) with pathogenic potential, increasing environmental stress of infection, while acting as a stressor in fish, causing the release of cortisol and decreasing the resistance, favoring the disease emergence (MORAES; MORAES, 2009).

Usually, coliform does not inhabit intestine of fish and when isolated from the gastrointestinal tract from these animals, suggest inadequate microbiological condition of the water (LORENZON et al., 2010).

Since they are not part of fish, the coliform presence is associated to fecal contamination of water from the capture site or improper handling of fresh fish (FRAZIER; WESTHOFF, 1988).

The gill epithelium is sensitive to environmental variations, responding to the functional need by lesions in the tissue structure. The close contact to the water favors the influence of dissolved chemical compounds, leading to morphological alterations (PAWERT et al., 1998).

Pollutant effects on gill structure of fish taken from polluted environments or exposed to laboratory test provide an indication of water contamination (SCHWAIGER et al., 1997).

Tilapia caught from streams that receive untreated domestic wastewater present gills with hyperplasia and detachment of the epithelium from the filament and lamella, complete and incomplete fusion of lamella, hypertrophy and hyperplasia of chloride cells, hemorrhage with epithelial rupture and aneurysm (LUPI, 2006).

Winkaler et al. (2001) studying the lambari (*Astyanax jacuhiensis*) sampled at Capivara Stream, near the battery factory, and Cambé Stream, polluted by domestic wastewater, in Londrina County (Paraná State), severe gill lesions as aneurysm, rupture of pillar cells and dilation of blood vessels, epithelial lifting characterized by detached epithelium.

Fontaínhas-Fernandes et al. (2008) verified that adult tilapia exposed to wastewater from sewage treatment plant had edema with detachment of lamellar and filament epithelium and lamellar fusion, cell proliferation with thickening of gill filament.

In this way, the present study analyzed microbiologically the water and the structural gill lesions from Nile tilapia (*Oreochromis niloticus*) and hybrid tambacu (*Colossoma macropomum* female x *Piaractus mesopotamicus* male), from fish farming in Itapecuru-Mirim County, Maranhão State.

Material and methods

Sampling stations

Seven samplings were performed, two in March, one in May and other in June 2008 referring to rainy period and one at each of the months of September and November 2008 and January 2009, referring to dry period.

The samplings stations were the fish farming A, B, C, D, E and F whose geographical coordinates and pond types are presented in Table 1.

The fish farming A was composed by 60 net cages (2.5 x 3 x 1.5 m in depth) at Itapecuru-Mirim River (Maranhão State). Within each pond, 1,000 tilapia fingerlings were inserted with a feeding rate of 1.5 kg of diet, three times per day.

Table 1. Geographical coordinates and pond types of the fish farming in Itapecuru-Mirim County, Maranhão State.

Fish farming	Pond types	Latitude	Longitude
A	Net cage	3°25'41.28"S	44°23'14.60" W
B	Net cage	3°23'32.33"S	44°21'39.92"W
C	Ground pond	3°28'33.2"S	44°21'01.5"W
D	Ground pond	3°19'09"S	44°21'08.4"W
E	Ground pond	3°23'44.1"S	44°24'06.3"W
F	Ground pond	3°28'42.4"S	44°21'35.5"W

The fish farming B was compounded by 12 net cages (2.5 x 3 x 1.5 m in depth) Itapecuru-Mirim River (Maranhão State), with 800 tilapia fingerlings within each one. Five hundred grams of diet were furnished, four times per day, and this amount increased gradually according to fish growth.

The fish farming C reared tilapia at six ground ponds (100 x 30/1.80 m in depth), containing 5,000 fingerlings each one. The feeding was made with 1 kg of diet three times per day and for the adults 3.5 kg per pond.

The fish farming D reared tambacu at four ground ponds (20 x 30/3 m in depth); 3,000 fingerlings were inserted at each pond, with feeding of 50 - 100 g every 2h for fingerlings. For the adults, 4.5 kg of diet, three times per day for each pond.

The fish farming E reared tambacu at three ground ponds (100 x 22/3 m in depth), with 3,000 fingerlings within each. The feeding was 50 - 100 g every 2h. The adults received 4.5 kg of diet, three times per day for each pond.

The fish farming F reared tambacu at 11 ground ponds (20 x 30/1.50 m in depth), with 1,000 fingerlings within each. The feeding followed the Purina table, according to the size, weight and fish quantity.

Samplings

We captured 57 adult fish using fishing net: 32 Nile tilapia and 25 hybrid tambacu, at fish farming employing net cages in the lower stretch from Itapecuru river (Maranhão State) and at ground ponds located in the same county, supplied by water from this river. In these farms, the monoculture prevails and the rearing of tilapia and tambacu predominate as subsistence activity to the local community. In the Table 2, we present the location, month and quantity of fish caught at each fish farming.

Table 2. Sampling month and number of tilapia and tambacu taken from each fish farming in Itapecuru-Mirim County, Maranhão State.

Fish farming	Month	Fish
A	March and November	10 tilapia
B	March and November	10 tilapia
C	May and September	12 tilapia
D	September and January	8 tambacu
E	June and November	10 tambacu
F	May and January	7 tambacu

Histological evaluation

After macroscopical analysis, gill fragments were collected and fixed (formalin 10%) for paraffin embedding and staining with hematoxylin and eosin (LUNA, 1968).

Microbiological evaluation

The methodology consisted in the weighing of 10 g of gills and the addition of 90 mL of peptone water (0.1%), and from this initial dilution, we prepared the other dilutions (10^{-2} and 10^{-3}). Then, an aliquot of 1mL of each dilution was poured into sterile Petri dish and the standard count agar was spilled over (PCA). The Petri dishes were incubated using a bacteriological incubator at 35ºC, and after 24-48h, we counted the colonies and performed the Gram stain to characterize the microbiota, Gram positive or Gram negative (BRASIL, 1987). The samples of water from the fish farming were taken using sterile flasks (500 mL), kept into a cooler box containing ice and transported to the Laboratório de Alimentos e Água from the Curso de Medicina Veterinária. The microbiological parameters were analyzed according to the methodology recommended by Apha (1995, 2005).

Physico-chemical evaluation of the water

We examined the pH, turbidity, nitrate and phosphate of the water from fish farming in the Laboratório de Físico-química da Universidade Estadual do Maranhão. Some parameters followed the methodology by Silva and Oliveira (2001).

Statistical analysis

In order to examine the influence of the year period on the occurrence of gill lesions, as well as the pond type, fish species and sampling stations, we applied the Fisher's Exact test, at 5% probability (PIMENTEL-GOMES, 2000).

Results and discussion

Microbiological quality of fish farming water

The results from the microbiological analysis of the water are presented in the Table 3. In all fish farming, we observed the presence of total coliforms, *Escherichia coli* and heterotrophic bacteria, and even without Conama standards considering the population size of total coliform, the counting of these microorganisms at fish farming characterizes the water with excess of organic matter that could be harmful for the rearing. However, only the fish farming D presented *E. coli* above 1,000 coliform 100 mL^{-1} in sample taken during the dry period. Then, probably, there is some point source of pollution, such as sewage discharge, which increases the quantity of nutrients in the water, favoring the bacterial proliferation. This result is in disagreement with the Conama 413/2009 (CONAMA, 2009) that establishes that the counting of coliform does not exceed 1,000 100 mL^{-1} of freshwater sample (Class 2) destined to fish farming. The presence of these bacteria is probably related to the untreated sewage discharge directly into the river that supplies the fish farming. Our results corroborate those from Lorenzon et al. (2010) that found total coliform, thermotolerant, in tilapia in the water from fish-and-pay located in the micro basin from Rico Stream (São Paulo State), the most probable number (MPN) of total coliform in the rearing water ranged from 4.2×10^4 to 2.4×10^5 MPN 100 mL^{-1} and of thermotolerant coliform varied between 3.8×10^2 and 2.0×10^4 MPN 100 mL^1. Thus, the water quality should be monitored and contamination control measures are required to minimize the risk of disease transmit by the consumption of produced fish.

Furthermore, the poor water quality, beyond contaminating the fish and generate risk to the consumers, may stress the fish, making them more susceptible to infections (BELO et al., 2005; MORAES; MORAES, 2009).

Table 3. Microbiological values of the water from fish farming in Itapecuru-Mirim County, Maranhão State, at different sampling stations during rainy and dry periods, expressed in most probable number (MPN)*.

Fish farming	Rainy period			Dry period		
	Total coliform	*Escherichia coli*	Heterotrophic bacteria	Total coliform	*Escherichia coli*	Heterotrophic bacteria
A (NC)	173.3	111.8	>738	>2419.6	290.9	355
B (NC)	>2419.6	396.18	623	>2419.6	387.3	>738
C (GP)	24.8	2.0	507	>2419.6	182.9	>738
D (GP)	>2419.6	25.4	339	>2419.6	>2419.6	507
E (GP)	>2419.6	235.9	555	>2419.6	98.5	355
F (GP)	2419.6	2.0	440	2419.6	8.4	>738

*MPN (most probable number 100 mL^{-1} of sample), NG (net cage) and GP (ground pond).

Physico-chemical water quality

The quality of the water from the fish farming in relation to the physical and chemical parameters are presented in the Table 4.

Most fish farming presented turbidity within the standard established for Class 2 waters by the Conama Resolution (CONAMA, 2009), which should be up to 100 TU. Meanwhile, in the water in the fish farming D and E, the value was high, reaching 472.7 and 160.0, respectively, during the dry period. The high turbidity found impairs the light penetration in the water and reduce the photosynthesis from the submerged rooted vegetation and algae. This reduced development of plants may suppress the fish productivity. Therefore, turbidity may interfere on aquatic biological communities.

The water pH from fish farming E was 5.48 during the dry period and this value is in disagreement with Conama Resolution 2009 that establishes values ranging from 6 to 9, indicating the suitability for fish farming. The value found in that fish farming was below the limit and may provoke stress on the fish, predisposing them to infectious and parasitic diseases.

The metabolic activity of the fish or other aquatic animals produce acid that, within closed systems as the culture ponds, gradually accumulate, reducing the pH and when equal to 5.5. is potentially stressful and if lower, is lethal (MORAES; MARTINS, 2004).

The nitrogen and the phosphorus are essential to aquatic life and are an important index of the presence of recent organic discharge when at high concentrations. Phosphate values in the water, from fish farming A, B, D, E and F during the rainy period, and C, D and F in the dry period, do not match to the Conama Resolution 2009, since the values were high and the acceptable value is 0.025 mg L^{-1}. The presence of phosphate associated to high counting of *E. coli* in the water from fish farming D may be explained by the discharge of domestic sewage, mainly detergents, as well as possible leaching of the soil that carries pesticides and animal waste for the water bodies.

Gills microbiology

The Gram stain of bacteria from 32 fragment of tilapia gill and 25 of tambacu indicated that among the bacterial colonies, there was percentage of Gram-positive cocci greater than 40.62%, followed by Gram-negative cocci with 25%, Gram-positive rods with 21.87%, Gram-negative rods with 6.25% and Gram-positive diplococci with 6.25% in the tilapia gills. On the other hand, in the tambacu, the Gram-positive cocci presented 52%, followed by Gram-negative cocci with 20%, Gram-positive rods with 20% and Gram-negative rods with 8%. Moreover, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio anguillarum* and *Edwardsiella tarda* are considered opportunistic, belonging to the microbiota of the water, skin, gill and intestine of the fish. The unbalance in the environment-bacteria-host system may cause epizootic diseases (BARJA; ESTEVES, 1988). The Gram-negative rods and Gram-positive cocci found suggest the presence of at least some of these bacteria.

Histopathological analysis

The Table 5 indicate that there was no significant difference when comparing the different type of gill lesions in relation to the rainy or dry periods, evidencing that the lesions are independent of the period.

The correlation between the lesions and pond type pointed significant difference ($p < 0.05$) for lamellar fusion and interlamellar hyperplasia, which presented greater incidence at ground pond (Table 6). The gill epithelium is the main contact surface with the environment and important target of water pollutants due to the extensive surface that increase the susceptibility to tissue lesions.

These nonspecific lesions act as defense mechanisms of the organ (ERKMEN; KOLANKAYA, 2000; KARLSSON-NORRGREN et al. 1985), as response for several type of toxic and vituperative agent and may impair the gill function.

Table 4. Physical and chemical values of the water from the fish farming in Itapecuru-Mirim County, Maranhão State, at different sampling stations during rainy and dry periods.

Fish farming	Rainy period				Dry period			
	Turbidity (TU)	pH	Nitrate (mg L^{-1})	Phosphate (mg L^{-1})	Turbidity (TU)	pH	Nitrate (mg L^{-1})	Phosphate (mg L^{-1})
A	23.5	6.75	0.000	0.13	7.8	6.54	0.016	0.018
B	22.6	6.15	0.000	0.19	8.6	7.4	0.039	0.005
C	7.8	7.12	0.000	0.01	38.1	6.8	0.353	0.117
D	58.3	6.81	0.2	0.26	472.7	6.82	2.046	0.082
E	45.6	6.56	0.1	0.13	160.0	5.48	0.096	0.014
F	0.0	7.23	3.9	0.03	24.2	6.01	0.097	0.075

Mallatt (1985) and Winkaler et al. (2001) and Fontaínhas-Fernandes et al. (2008) observed that, when exposed to toxic substances, the gills presented lesions, such as epithelial lifting, necrosis, hypertrophy, hyperplasia, lamellar fusion, rupture of gill tissue, hypersecretion and proliferation of mucosal cells. Thophon et al. (2003) refer to the presence of edema accompanied by the detachment of lamellar epithelium as the first sign of severe gill lesion.

Table 5. Occurrence of gills lesions in fish sampled during the rainy and dry period in Itapecuru-Mirim County, Maranhão State.

Gill lesions	Absence (A) Presence (P)	Year periods		Probability
		Rainy	Dry	
Lamellar fusion	A	12 (21.05%)	16 (28.07%)	0.13[ns]
	P	16 (28.07%)	13 (22.81%)	
Interlamellar hyperplasia	A	14(24.56%)	20 (35.09%)	0.07[ns]
	P	14 (24.56%)	9 (15.79%)	
Sub-epithelial edema	A	23 (40.35%)	25 (43.86%)	0.25[ns]
	P	5 (8.77%)	4 (7.02%)	
Telangiectasia	A	20 (35.09%)	18 (31.58%)	0.16[ns]
	P	8 (14.04%)	11 (19.30%)	

ns - non-significant at the level of 5% of probability.

Table 6. Occurrence of lesions in fish gills related to the pond type in Itapecuru-Mirim County, Maranhão State.

Gill lesions	Absence (A) Presence (P)	Pond type		Probability
		Ground pond	Net cage	
Lamellar fusion	A	14 (24.56%)	14 (24.56%)	0.01*
	P	23 (40.35%)	6 (10.53%)	
Interlamellar hyperplasia	A	25 (43.86%)	9 (15.79%)	0.005*
	P	12 (21.05%)	11 (19.30%)	
Sub-epithelial edema	A	33 (57.89%)	15 (26.32%)	0.11[ns]
	P	4 (7.02%)	5 (8.77%)	
Telangiectasia	A	26 (45.61%)	12 (21.05%)	0.16[ns]
	P	11 (19.30%)	8 (14.04%)	

ns - non-significant at the level of 5% of probability, *significant at the level of 5% of probability.

Considering the occurrence of lesions in the different fish species, the statistical analysis evidenced significant difference ($p < 0.05$). The tambacu was more sensitive to lamellar fusion, while in tilapia, the other lesions presented higher incidence (Table 7). This difference may be only casual, since there is no justification.

Table 7. Occurrence of lesions in fish gills related to the fish species from fish farming in Itapecuru-Mirim County, Maranhão State.

Gill lesions	Absence (A) Presence (P)	Fish species		Probability
		Tambacu	Tilapia	
Lamellar fusion	A	7 (12.28%)	21 (36.84%)	0.0041*
	P	18 (31.58%)	11 (19.30%)	
Interlamellar hyperplasia	A	21 (36.84%)	13 (22.81%)	0.00082*
	P	4 (7.02%)	19 (33.33%)	
Sub-epithelial edema	A	24 (42.11%)	24 (42.11%)	0.0292*
	P	1 (1.75%)	8 (14.04%)	
Telangiectasia	A	21 (36.84%)	17 (29.82%)	0.0112*
	P	4 (7.02%)	15 (26.32%)	

*significant at the level of 5% of probability.

Regarding the sampling stations, there was statistical difference for all gill lesions (Table 8). The lamellar fusion presented higher occurrence in the fish from the fish farming E and F, intermediary in C and D and lower in A and B.

The interlamellar hyperplasia was more frequent in the fish farming A and C, intermediary in B and lower in D and F, and absent at E.

The sub-epithelial edema presented greater occurrence in the fish farming A, intermediary in C, was lower in B and D, and absent in E and F.

The telangiectasia had greater occurrence in the fish farming C, intermediary in B, lower in A, D and E and absent at F.

Regarding the significant difference in the gill lesions between the species and the different fish farming, we recorded that the tambacu reared at ground pond in the fish farming E and F was more sensitive to lamellar fusion; besides that, the tilapia reared in the fish farming A (net cages) and C (ground pond), to interlamellar hyperplasia; at fish farming A, the predominant lesion was the sub-epithelial edema and at fish farming C, telangiectasia.

The tambacu that were reared in ground pond in the fish farming D and E developed interlamellar hyperplasia and, consequently, lamellar fusion that might have occurred due to the excess of particulate matter as verified through the high turbidity. On the other hand, the tilapia reared in net cages in the fish farming A developed interlamellar hyperplasia and sub-epithelial edema and at fish farming C occurred interlamellar hyperplasia and telangiectasia in the tilapia reared in ground pond, as a result of not only particulate matter but also by the high content of phosphate.

The presence of telangiectasia in the fish farming may have been the result of the rupture of pillar cells, which allow the dilation of sinusoidal capillary of lamella and may have been associated to physical or chemical trauma. Roberts (1981) argues that this lesion occurs after more severe handling and may be related to parasitic lesion, metabolic waste or chemical contaminants and when many lamellae are affected, the respiratory function may decrease mainly at high temperature, when the oxygen levels are low and the metabolic demand is high. If the fish are traumatized, the gills may rupture and then the fish die by hemorrhage.

In the present study, the lesions predominating in the gills were: lamellar fusion by the hyperplasia (Figure 1b), sub-epithelial edema (Figure 1c) and telangiectasia (Figure 1d).

Table 8. Occurrence of lesions in fish gills related to the sampling station in Itapecuru-Mirim County, Maranhão State.

Gill lesions	A P	A	B	C	D	E	F	P
Lamellar fusion	A	7 12.28%	7 12.28%	7 12.28%	4 7.02%	0 0.00%	3 5.26%	p < 0.05
	P	3 5.26%	3 5.26%	5 8.77%	4 7.02%	7 12.28%	7 12.28%	p < 0.05
Interlamellar hyperplasia	A	2 3.51%	7 12.28%	4 7.02%	6 10.53%	7 12.28%	8 14.04%	p < 0.05
	P	8 14.04%	3 5.26%	8 14.04%	2 3.51%	0 0.00%	2 3.51%	p < 0.05
Sub-epithelial edema	A	6 10.53%	9 15.79%	9 15.79%	7 12.28%	7 12.28%	10 17.54%	p < 0.05
	P	4 7.02%	1 1.75%	3 5.26%	1 1.75%	0 0.00%	0 0.00%	p < 0.05
Telangiectasia	A	7 12.28%	5 8.77%	5 8.77%	5 8.77%	6 10.53%	10 17.54%	p < 0.05
	P	3 5.26%	5 8.77%	7 12.28%	3 5.26%	1 1.75%	0 0.00%	p < 0.05

*significant at the level of 5% of probability, A-absence and P-presence, P-probability.

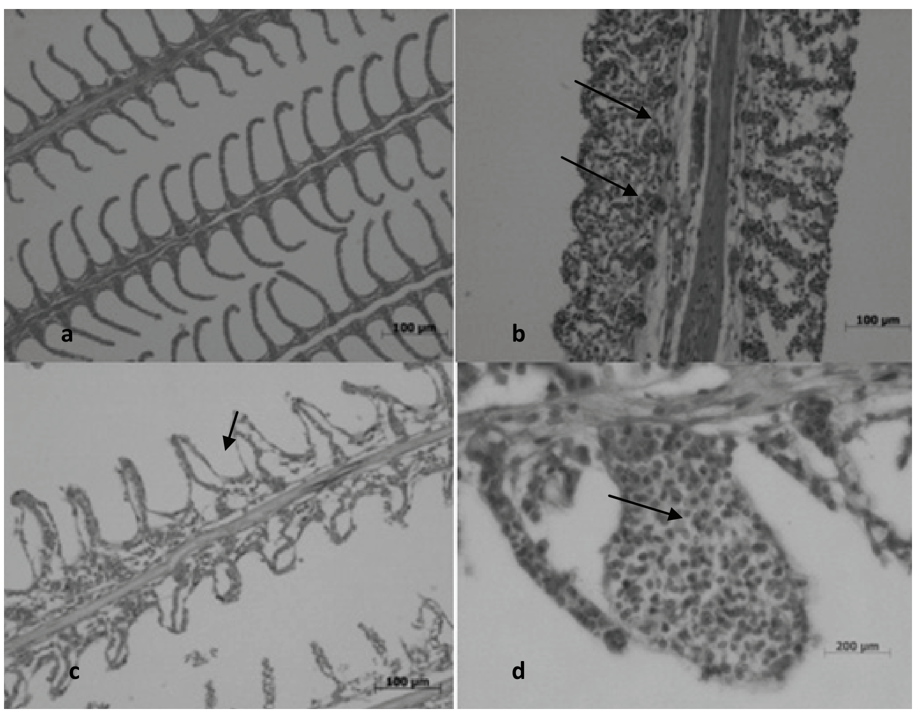

Figure 1. (a) Tambacu gills presenting normal morphology, H.E, 40x, (b) Tilapia gills (*Oreochromis niloticus)* evidencing the epithelial hyperplasia and lamellar fusion (arrows), H.E, 40x, (c) Tilapia gills (*O. niloticus*) showing epithelial detachment probably due to the sub-epithelial edema (arrow), H.E,40x and (d) Tilapia gills (*O. niloticus*) presenting telangiectasia (arrow l), H.E, 100x.

Conclusion

The present study suggests that the observed lesions were hyperplastic and circulatory in nature and may be an excess of particulate matter or chemical pollutants at the water. As we did not detect lesions of inflammatory nature, at least in the examined fish, gill infections were not verified despite the polluted environment.

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