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Ecomonitoring of Climate Impact on *Tilapia niloticus* Performance and Development of Different Histopathological Changes

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Abstract: Monitoring water quality was so important to assess and mange the risk associated with climate change impact and consequent stress. Assessing seasonal impact on aquaculture water quality parameters and fish performance (final body weight and organosomatic indices) was estimated in 10 earthen ponds aquaculture in ALFayoum province. Water and fish samples were collected during summer, spring and autumn 2009. Biomarkers as organs-somatic indices (SI) and histopathological alterations were determined. Results revealed that, spring season characterized by significant differences in mean values of water quality parameters Vs autumn and summer with absence of seasonal significant differences in dissolved oxygen (DO) values. Significant differences were recorded in final body weight (FBW), organs-somatic indices (SI) and tissue lesions in all examined vital organs. Histopathological investigation during winter (December and January) revealed, gill arch showed dense aggregation of eosinophilic granular cells (EGCs) with leucocytes infiltration and edematous fluid exudation. The gill filaments showed lamellar hyperplasia with fusion of secondary gill lamellae and proliferation of lamellar epithelium. Fish gills were among the most recognized organs affected by water quality changes. Liver showed small foci of vacuolar degeneration of hepatocytes with small multifocal aggregation of mononuclear cells. Diffuse hepatocellular degeneration with congestion of hepatoportal blood vessel. Spleen showed lymphoid depletion with congestion of sinusoids and necrosis of the splenic ellipsoid. Spleen tissue showed individual case of encysted metacercaria. Brain edema, congestion of cerebral blood capillaries, neuronal degeneration and necrosis were the most oblivious brain tissue alterations. It's recommended to examine water periodically and randomly harvest fish for rapid evaluation of affected fish biomarkers. Owners must be alert to the expected seasonal alteration on the water quality of earthen ponds aquaculture from the socio-economic aspect.

Key words: Water quality · Seasonal change · Biomarkers · Histological alterations · Earthen ponds, Organosomatic indices

INTRODUCTION

Managing the water used for aquaculture is one of the most essential components for aquaculture system to compatible with the requirements of the fish being held with regard to ammonia, nitrite, nitrate (in marine systems), pH, temperature, DO, hardness, alkalinity and salinity. Monitoring the water quality frequently to reduce the risks associated with stress and disease. Tilapia culture has been carried out in earthen pond, monoculture, polyculture and in different environment (fresh water and saline water) [1-3]. One of the great advantages of using histopathological biomarkers in environmental monitoring is to examine specific target vital organs, including gills and liver for excretion and the accumulation and biotransformation of xenobiotics in the fish. These biomarkers were closely related to other biomarkers of stress since many pollutants had to undergo metabolic activation in order to be able to provoke cellular change in the affected organism [4,5]. Qualitative histology-based assessment, fish necropsy and organosomatic indices were recorded and the histological alterations was assessed using a scale such as mild, moderate and severe [6]. The daily and seasonal changes in temperature are

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challenges that fish within aquaculture settings cannot completely avoid and are known to elicit complex organismal and cellular stress responses [7]. In freshwater fishes, ammonia was thought to traverse the gill epithelium almost exclusively via passive diffusion of ammonia as a gas in solution. The suspended solids in freshwater may clog fish gills. The gills may be injured by turbid water. Although the effect will depend on the species and the nature of the suspended matter, pronounced effects were seen when the water contains about 4 percent by volume of solids. Exposure to 0.33 mg unionized ammonia-nitrogen / liter (UIA-N^{-1})induced gill damage and subsequent liver tissue hypoxia associated with blood anemia, [8-10].An increased number of macrophages aggregates could be found in the liver and spleen in fish exposed to chemical pollutants, bacteria, fungi or parasites. Liver was the main detoxification organ involved in the metabolism and excretion of xenobiotic chemicals. Liver necrosis had been shown in Nile Tilapia (*O. niloticus*) after exposure to sediment containing a variety of organic chemicals [11-13].

The current field investigation was conducted for monitoring the water quality used in earthen pond fish aquaculture in different seasons in AL Fayoum governorate, Egypt and its impact on some of fish performance parameters.

MATERIALS AND METHODS

Sample Collection for Water Quality Analytical Methods: Water samples (160 totals) were collected from the ponds biweekly (two times monthly) between 12.00 and 02.00 h pm during April to November 2009. Water temperature, DO and pH were measured on site. Water samples were placed in 1-L plastic bottles and transported to the laboratory for chemical analytical examination, while glass bottles 150 ml were used for microbiological examination and kept in ice tank till reach lab [14].

Collection and Preservation of Samples: The obtained samples were met the requirements of the sampling program and were handled so that they did not deteriorate or contaminate before they were analyzed. Composite representative samples were collected from ten different earthen ponds at different points and depths.

Microbiological Analysis of Water Samples: Clean, dry, screw capped glass bottles of 150 ml capacity were used for collecting the water samples. The bottles were sterilized in hot air oven at 170° C for 60 min. Changes caused by growth of microorganisms were greatly retarded by keeping the sample in refrigerator at a low temperature (< 4°C but above freezing).

Physical and Chemical Analysis of Water Samples: These analysis were done after APHA, [15]. Temperature, of water samples was measured at the time of sampling by means of an ordinary thermometer (range 0-100 °C). pH, values of water samples were determined by means of electrometric pH meter (pHep® HI 98107. Italy). Dissolved Oxygen (DO), was measured by (Membrane Electrode Method) through using portable waterproof dissolved oxygen meter (HI 9142. Italy) [16].

Total Hardness: Ethylene Damine Tetraacetic Acid (EDTA)Titrimetric Method. Total Solids measure was done after APHA, [15]. Total Dissolved Solids (TDS) were measured by using waterproof EC/TDS/NaCl/°C Meter (HI 9835. Italy).Total Suspended Solids (TSS), were obtained by calculation of the difference between total dissolved solids and total solids. TSS (g/L) = TS-TDS. Electrical Conductivity (EC) and Salinity (NaCl %), EC was measured by using waterproof EC/TDS/NaCl/°C Meter (HI 9835. Italy) NaCl Calibration, the HI 7037 calibration solution (sea water solution) was used as 100% NaCl % standard.Chloride was determined by using Argentometric method. Total alkalinity was determined by Potentiometric titration to end-point pH [15].

Organic matter, Chemical oxygen demand (COD) was measured using the 'heat-of-dilution' dichromate oxidation method [17]. Ammonia was determined by using (Direct Nesslerization Method). Nitrite (NO_2) was determined by using colorimetric method, Phosphates were determined by using the stannous chloride colorimetric method [15].

Heterotrophic plate count (HPC, The standard plate count) was determined by Pour plate method after APHA, [15]. Total fungal count of water (TFC, CFU/ ml) was determined by using Pour Plate Technique and Sabouraud Dextrose Agar. (Oxoid, Code: CM0041).

Standard R-Total Coliform Fermentation Technique (T Coliform.C): T. Coliform. C was determined by using multiple tube fermentation technique [15]. The presumptive and confirmed phases of the multiple-tube procedure were used for non potable water samples.

Evaluation of Some Fish Performance Parameters: A total 100 randomly selected fish at end of rearing were collected for estimating of:

- Fish body size and weight (BS and BW) to the nearest cm² and gram respectively [18].
- Organosomatic indices; immediately after dissection was made, the liver, spleen were removed and weighed for calculating their weights and somatic indices. Organosomatic indices are ratios of organ weight to body weight and have been used in various stress-related studies [19]. Hepatosomatic indices were employed to support the findings of the qualitative and quantitative histological assessment of liver tissue [20].
- Histopathological Investigation.

Samples were collected from gills, liver, spleen and brain of the tilapia fish and fixed in the 10% buffered formalin for 24 h, dehydrate through a graded series of ethanol and clear with xylene solutions. They were embedded in a block using melted paraffin. The paraffin blocks were sectioned at 4-5 ìm thickness using a rotary microtome and stained with hematoxylin and eosin according to Bancroft and David, [21].

Statistical Analysis: Descriptive and analytical tests were carried out using Student "t" Test to compare between the results of physicochemical character and microbial load within ponds and in between ponds during seasons of the study. Pearson 2-tailed correlation test is used [22].

RESULTS AND DISCUSSION

Data shown in Table (1) indicated highly significant decrease ($P \leq 0.001$) in temperature mean values between spring Vs summer and also between autumn Vs spring and summer. No significant difference were noticed in DO mean values between seasons. Chloride was significantly decreased ($P = 0.01$) in spring Vs summer while highly significant decreased ($P \leq 0.001$) Vs autumn. Hardness was significantly decreased ($P \leq 0.01$) in spring Vs summer while highly significant decrease ($P \leq 0.001$) was recorded Vs autumn. The pH mean values demonstrated highly significant decrease ($P \leq 0.001$) between spring Vs summer and autumn and also between summer Vs autumn.

Table 1: Seasonal Seasonal Significant differences of physicochemical characters of the examined water ponds

	Spring		Summer		Autumn	
Season Parameter	Summer	Autumn	Autumn	Spring	Spring	Summer
Temperature C°	-2.49***	5.23***	2.49***	7.72***	-5.22***	-7.72***
	0.000	0.000	0.000	0.000	0.000	0.000
Dissolved oxygen ppm	-0.46	-0.54	0.46	-0.08	0.54	0.08
	0.27	0.25	0.27	0.86	0.24	0.86
Chloride mg/L	-734.25**	-1153.66***	734.2**	-419.41	1153.6***	419.41
	0.01	0.000	0.01	0.17	0.000	0.17
Hardness mg/L	-375.50**	-531.01***	375.5**	-155.51	531.01***	155.51
	0.01	0.000	0.01	0.32	0.000	0.33
PH	-.31***	-.60***	.31***	-.29***	.60***	.29***
	0.000	0.000	0.000	0.000	0.000	0.000
Alkalinity mg/L	-49.68**	-228.46***	49.68**	-178.78***	228.46***	178.78***
	0.01	0.000	0.01	0.000	0.000	0.000
NO_2-N mg/L	0.02	-0.02	-0.02	-0.04	0.02	0.04
	0.37	0.46	0.37	0.12	0.46	0.12
NH_3-N mg/L	-0.15	-.34***	0.15	-0.19	.34***	0.19
	0.15	0.000	0.15	0.11	0.000	0.11
PO_4 mg/L	-0.8	-2.45*	0.8	-1.65	2.45*	1.65
	0.41	0.03	0.41	0.13	0.03	0.13
Electrical Conductivity ms/cm	-1.49*	-2.37***	1.49*	-0.88	2.37***	0.88
	0.04	0.000	0.04	0.27	0.000	0.27
Total solids g/L	-1.48	-2.40**	1.48	-0.92	2.40**	0.92
	0.08	0.01	0.08	0.33	0.01	0.33
Total suspendsd solid (g/L)	-0.74	-1.21*	0.74	-0.47	1.21*	0.47
	0.13	0.03	0.13	0.39	0.03	0.39
Total dissolved solid g/L	-.75*	-1.19***	.75*	-0.44	1.19***	0.44
	0.04	0.000	0.04	0.27	0.000	0.27
NaCl %	-2.89*	-4.69***	2.89*	-1.8	4.69***	1.8
	0.04	0.000	0.04	0.26	0.000	0.26
COD mgO_2/L	-0.35	-8.55***	0.35	-8.19***	8.55***	8.19***
	0.87	0.000	0.87	0.000	0.000	0.000

*** Highly significant at $P \leq 0.001$ ** significant at P=0.01

* Low significant at $P \leq 0.05$.

Table 2: Mean values ± SE of some fish performance indices in studied ponds

No. of pond	Body size BS/ (cm^2)	Final body weight. FBW/g	Liver weight LW/g	Liver Somatic Index LSI	Spleen weight SW/g	Spleen Somatic Index SSI
1	185.9± 7.69	239.82± 8.27	3.79± 1.35	1.61± 0.59	0.53±0.16	0.23±0.07
2	137.4± 2.76	148.85± 2.34	2.48± 0.11	1.67±0.10	0.27±0.02	0.18±0.02
3	99.2±4.56	78.48±5.49	0.63±0.04	0.81±0.01	0.09±0.01	0.11±0.003
4	100.6±4.44	80.69±5.02	0.64±0.03	0.80±0.01	0.09±0.01	0.11±0.004
5	93.2±2.97	71.08±3.21	0.58±0.02	0.82±0.003	0.07±0.01	0.10±0.004
6	96.3±2.20	74.70± 2.38	0.61±0.02	0.81±0.003	0.08±0.003	0.11±0.002
7	204.7±3.98	295.00±6.01	4.16±0.49	1.43±0.20	0.21±0.03	0.07±0.01
8	197.6±4.65	309.38±4.07	3.67±0.22	1.19±0.08	0.39±0.06	0.12±0.02
9	169.2±6.34	200.50±7.78	3.89±0.03	1.95±0.06	0.25±0.04	0.12±0.01
10	156.0±16.01	193.20±25.86	3.16±0.33	1.67±0.08	0.37±0.10	0.18±0.03
All ponds	144.01±6.34	169.17±12.84	2.36±0.25	1.28±0.08	0.23±0.03	0.13±0.01

Alkalinity decreased significantly (P = 0.01) during spring Vs summer while highly significant increase (P ≤ 0.001) was recorded between Autumn Vs spring and summer. No significant difference was noticed in Nitrite mean values between seasons. Highly significant decrease (P ≤ 0.001) in total ammonia nitrogen (TAN) mean values was recorded between spring Vs autumn. PO4+ concentration was less significantly decreased (P = 0.03) during spring in comparison to autumn. EC was less significantly decreased during spring Vs summer (P = 0.04) while highly significant decrease (P ≤ 0.001) was recorded Vs autumn. TS were significantly decreased during spring Vs autumn (P = 0.01). TSS were significantly decreased (P = 0.03) during spring Vs autumn. TDS were significantly decreased during spring Vs summer (P = 0.04) while highly significant decrease (P ≤ 0.001) was recorded between spring Vs Autumn. NaCl % was significantly decreased during spring Vs summer (P = 0.04) while highly significant decrease was recorded during spring Vs autumn (P ≤ 0.001). Highly significant increase in mean values of COD (P ≤ 0.001) was recorded between Autumn Vs spring and summer. From the above mentioned data it's clear that the spring season characterized by the significant differences in the mean values of TS, TSS, TDS, EC., Alk., PH, PO_4^-, TAN, Salinity, COD, Hd and Chlorides Vs autumn and summer.The absence of significant differences of mean DO values between seasons might be due to the absence of aerators in 8/10 of ponds with minimizing surface water movement and the subsequent decreased mixing air O_2 into these ponds water. The higher TAN values recorded during hot period, might be attributed to the elevation of the water temperature and the increase in the evaporation rates and the accumulation of the dissolved salts in water. The relative increase in the ammonia during hot period may be attributed to the high evaporation rate, in addition to the denitrification process by the reduction of NO2-and NO3-into NH3 [23]. In addition to temperature, oxygen solubility was also affected by salinity and impurities. Low dissolved oxygen in an aquaculture operation was at high concentration of biodegradable organic matter in the water which associated the high temperatures as demonstrated [24]. However, Tilapia can survive below 0.3 mg/L of DO and the aerator were so important to keep morning DO from falling below 0.7-0.8 mg/L when compared with non aerated ponds as reported [25].

Table (2): Illustrated the mean values ± SE of some fish performance parameters, the data clarified that the highest mean value of BS was 204.7 ± 3.98 cm² in pond no. 7, while the smallest value was 93.2 ± 2.97 cm² in pond no.5. The highest mean value of FBW was 309.38 ± 4.07 g. in pond no. 8, while the lowest mean value of FBW was 71.08 ± 3.21 g. in pond no. 5. The highest mean value of LW was 4.16 ± 0.49 g. in pond no. 7, while the lowest was 0.58 ± 0.02 in pond no. 5. The maximum mean value of LSI was 1.95 ± 0.06 in pond no. 9, while the minimum value was 0.80 ± 0.01in pond no. 4. The maximum mean value of SW was 0.53± 0.16 g. in pond no.1 while the minimum value of (SW) was 0.07 ± 0.01 g. in pond no. 5. The maximum mean value of SSI was 0.23 ± 0.07 in pond no.1,while the minimum was 0.07± 0.01in pond no.7. From the aforementioned data it is noticed that pond no.5 characterized by the smallest BS, lowest FBW, LW and SW. This pond had highest pH value during summer and autumn (8.30 and 8.80 respectively), these values may decrease mean FBW which attributed to the decreased feed consumption and consequent growth rate [26,27,28] No health or sub-lethal effects at ammonia range 0.0-0.3 mg/L, possible sub-lethal effects in warm-water fish occurred in the range of 0.3-0.8 mg NH_3 /L [29].

Table 3: Correlations between Body Weight, Body Size, Liver Somatic Index and Spleen Somatic Index

	Body size / cm^2	Final body weight /g	LSI	SSI
Body size (cm^2)	1	.981***	.431**	.172
		.000	.002	.232
Final Body Weight /g	.981***	1	.385**	.137
	.000		.006	.342
LSI	.431**	.385**	1	.622***
	.002	.006		.000
SSI	.172	.137	.622***	1
	.232	.342	.000	

*** Highly significant at $P \leq 0.001$

** significant at $P \leq 0.01$

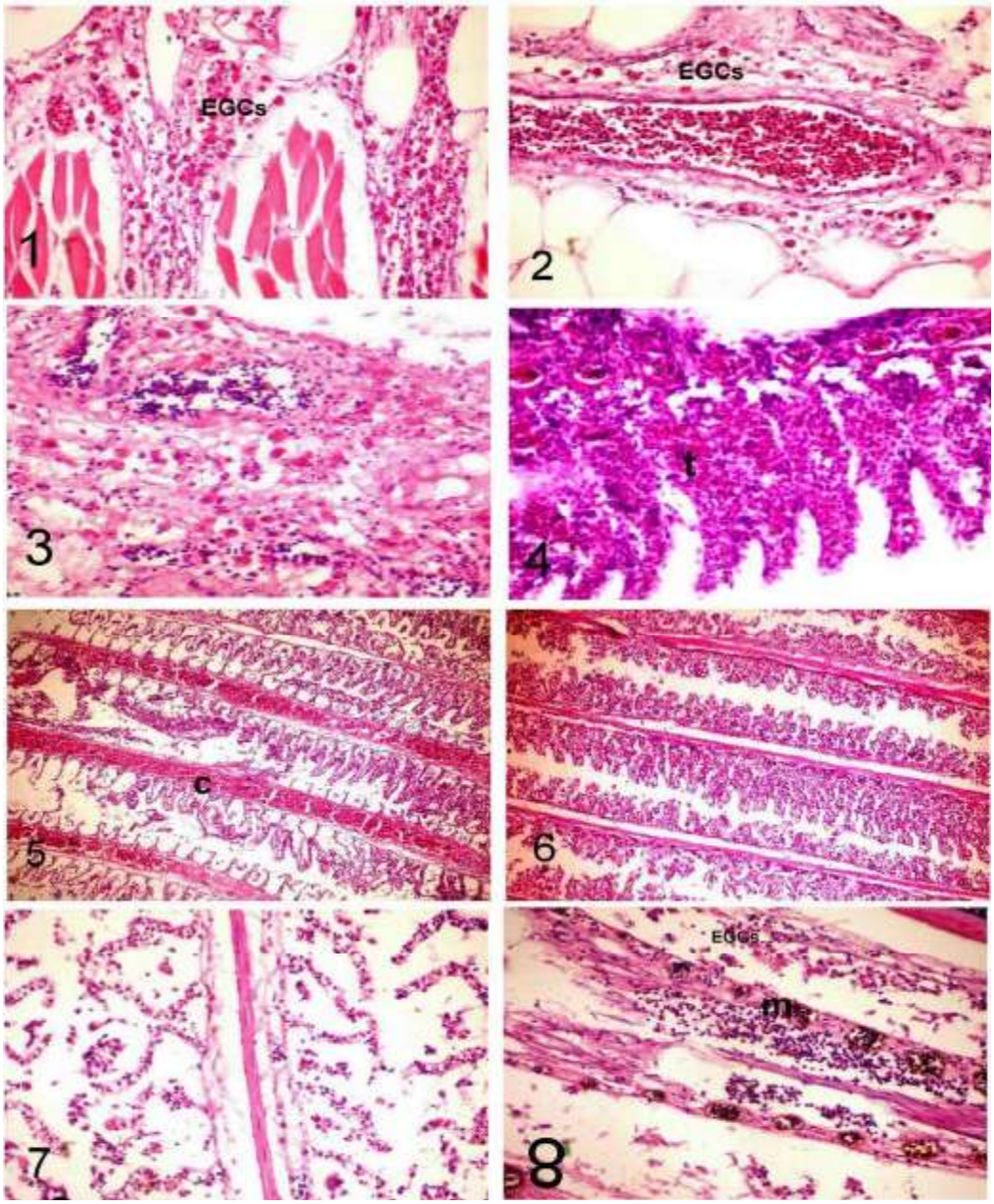
Table (3): Discussed the presence or absence of significant correlation between fish performance parameters (Pearson, 2-tailed).Highly significant correlation was recorded between BS and FBW and also between LSI and SSI at $P \leq 0.001$. LSI had significant correlation with SSI and FBW at $P \leq 0.01$. Many of water quality parameters are involved in decreased FBW, as increased pH (6-9), ammonia (mainly unionized form) [27,30], nitrite [31-33], increased salinity (in fresh water fish) [34,35], too low [36] or too high DO [37,38], increased EC, TS, TSS, TDS [39,40], all interfered with respiration and biochemical activities of fish as bubble formation in blood with increased losses.

Histopathological Findings: Histology and histopathology could be used as bio-monitoring tools or indicators of health in toxicity studies as they provide early warning signs of disease [41]. Histopathological alterations are biomarkers of effect exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function [42]. The micrographs of the examined fish organs are listed in legend 1(Fig.1-28): The examined fish organs collected from ten different earthen ponds under investigation during winter revealed the following:

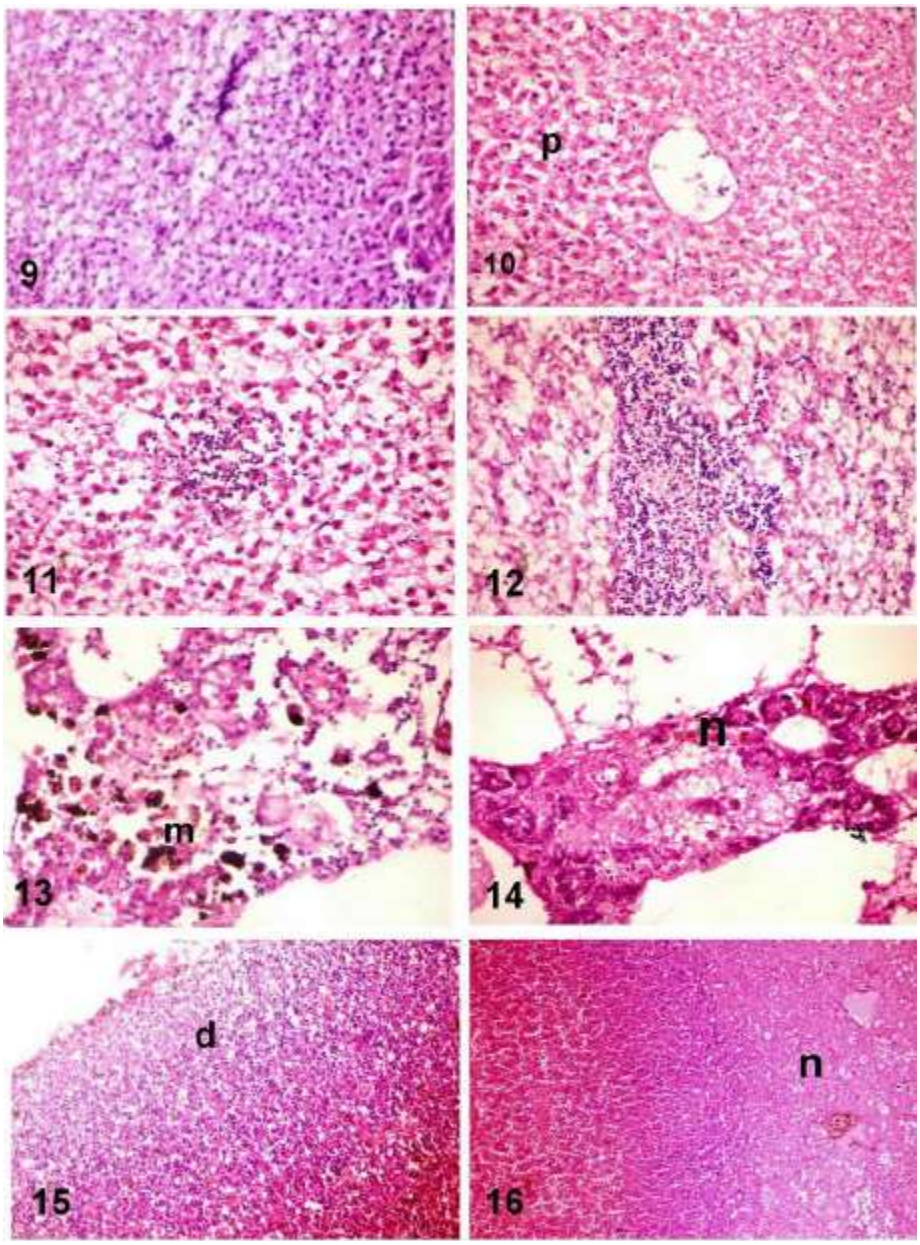
Gills: The microscopic examination of the Tilapia gills revealed various histopathological alterations that varied in severity. Concerning the gill arch (GA), dense aggregation of eosinophilic granular cells (EGCs) was observed between muscle bundles (Micrograph 1).The lesion associated with congestion of blood vessel with perivascular aggregation of EGCs (Micrograph 2) associated with leucocytes infiltration and edematous fluid exudation (Micrograph 3).The mentioned less severe lesions were recognized in fish from ponds no.1,2. These ponds were in wind direction and held monoculture (*Tilapia niloticus*) and obtained the fry from external hatchery. These ponds had used fertilizers, pond 2, had increased mean values of TFC and TCC.

The gill filaments (GF) showed variable lesions with varying degree of severity. Mild cases showed lamellar hyperplasia (L Hp) with fusion of secondary gill lamellae by the proliferation of lamellar epithelium (L Ep) (Micrograph 4). Areas of telangiectasis of lamellar blood capillaries (LBC) were characterized by dilatation of LBC (Micrograph 5).lamellar edema (LE) associated with congestion of main branchial blood vessel were observed (Micrograph 6). LHp associated with (LE) were also noticed (Micrograph 7).The lesions characterized by proliferation of LEp with edematous separation of lamellar epithelial cells (LEpCs) (Fig.8)., in severe cases of LE, the lesions was associated with necrosis and sloughing of (LEp) (Micrograph 9 and 10). The previously mentioned severe lesions were recognized in fish from ponds no. 3 and 4 associated with leucocytic infiltrations in GF and aggregation of Melanomacrophages Cells (MMCs) (Micrograph 11) and EGCs aggregation.

Pond no. 3 characterized by increased, ammonia, nitrite (autumn), TFC (spring). Temperature, T. Coliform. C (summer) and decreased (COD). The increased nitrogen contents in pond 3 exerts high oxygen demand for chemical which consumed and resulted in oxygen depletion [24]. Ponds no 3,4 had polyculture (Tilapia niloticus and Mugil), obtained the fry from their internal hatcheries and were located in opposite direction to the wind. These ponds did not use fertilizers. Pond no. 4 was characterized by increased T. Coliform. C. (summer). Increased TAN to toxic level mainly to gills. Histopathological effects, particularly those affecting gills function might contribute to reduce fish growth through inducing tissue hypoxia. [43]. High concentrations of TAN could cause gill damage, reduced the oxygen-carrying capacity of blood, increased the oxygen demand



Micrograph 1: Tilapia's gill arch showing dense aggregation of eosinophilic granular cells (EGC)(H and E 200 X).
Micrograph 2: Perivascular aggregation of EGCs with congestion of blood vessel(C) (H and E 400 X).
Micrograph 3: Tilapia's gill arch showing aggregation of EGCs with leucocytic infiltration and edematous fluid exudation (H and E 400 X)
Micrograph 4: Tilapia's gill filament showing lamellar telangiectasis (H and E400 X).
Micrograph 5: Tilapia's gill filament showing congestion of main branchial blood vessel (C) associated with lamellar edema (H and E 100 X).
Micrograph 6: Tilapia's gill filament showing lamellar hyperplasia associated with lamellar edema (H and E 200 X).
Micrograph 7: Tilapia's gill filament showing lamellar edema with edematous separation and sloughing of lamellar epithelium (H and E 400 X).
Micrograph 8: Tilapia's gill filament showing sloughing and necrosis of lamellar epithelium with leucocytic infiltration and melanomacrophage aggregation, EGCs aggregation (H and E 400 X)



Micrograph 9: Vacuolar degeneration of hepatocytes with individual hepatocellular necrosis and focal mononuclear cell aggregation.

Micrograph 10: Hepatocellular vacuolation and individual cell necrosis with pyknotic nuclei(arrow) (H and E 400 X).

Micrograph 11: Tilapia's liver showing dissociation of hepatocytes with individual hepatocellular necrosis and focal mononuclear cell aggregation (H and E 400 X).

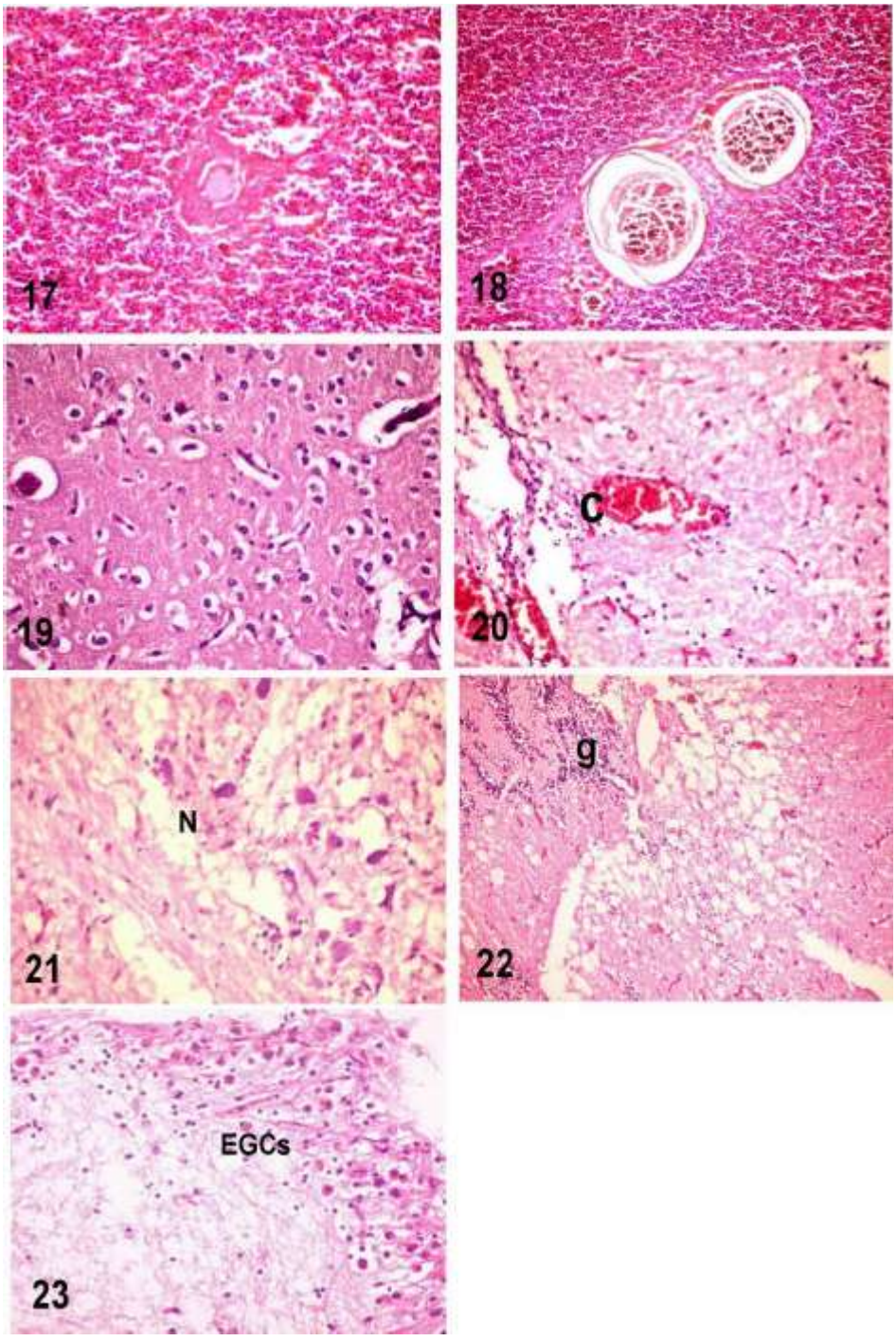
Micrograph 12: Tilapia's liver showing hepatocellular necrosis with loss of structural integrity and diffuse mononuclear cell infiltration.

Micrograph 13: Hepatocellular necrosis with MMCs aggregation with releasing of its content (melanin pigment) (arrow) (H and E 400 X).

Micrograph 14: Tilapia's liver showing necrosis and degranualtion of pancreatic acinar epithelium of hepatopancrease with MMCs aggregation (H and E 400 X).

Micrograph 15: Tilapia's spleen showing lymphoid depletion(arrow)(H and E 200 X).

Micrograph 16: Tilapia's spleen showing sub capsular necrosis (N) (H and E 200 X).



Micrograph 17: Tilapia's spleen showing necrosis of spleenic ellipsoids (arrow) (H and E 400 X).
Micrograph 18: Tilapia's spleen showing encysted metacercaria within splenic tissue. (H and E 200 X).
Micrograph 19: Tilapia's brain showing vacuolation of brain tissue (H and E 400 X).
Micrograph 20: Tilapia's brain showing congestion of cerebral blood vessel (C) associated with brain edema and gliossis.
Micrograph 21: Tilapia's brain showing edema, necrosis and demylination (H and E 400 X).
Micrograph 22: Tilapia's brain showing massive vacuolation of brain tissue with diffuse gliosis (H and E 200 X).
Micrograph 23: Tilapia's brain showing extensive aggregation of EGCs(arrow)(H and E 400 X).

of tissues, damage red blood cells and the tissues that produced them and affected osmoregulation[35]. Gills are the first target organ for waterborne pollutants due to the constant contact with the external environment, as well as the main place for copper uptake [44]. It is well known that changes in fish gill are among the most recognized responses to environmental pollutants [45.].When *Oreochromus niloticus* exposed to 5 µg/L deltamethrin revealed severe morphological alterations in the gills. In the gills hyperemia, fusion of secondary lamellae and telangiectasis were observed; whereas hydropic degenerations in liver were observed in all examined fish.[46].

Liver: Liver lesions varied from mild changes which characterized by small foci of vacuolar degeneration of hepatocytes (VD) with small focal aggregation of mononuclear cells (MCs) (Micrograph 12) as recognized in fish from ponds no. 7.8.9.10, to diffuse hepatocellular degeneration associated with congestion of hepatoportal blood vessel (Micrograph 13). kupffer cell aggregation associated with hepatocellular necrosis (HN) were noticed (Micrograph 14). Focal leucocytic aggregation associated with dissociation of hepatocytes and individual (HN) were also recorded(Micrograph 15). Perivascular leucocytic aggregation (Micrograph 16) and diffuse of (HN) with loss of structural integrity and infiltration of (MCs) were noticed (Micrograph 17). In individual cases the (HN) was associated with focal aggregation of melanin carrying cells (MMCs) (Micrograph 18) as recognized from fish in ponds no. 5,6,9. Concerning the hepatopancrease, the cases of severe (HN) was associated with necrosis of pancreatic acinar cells with (MMCs) infiltrating the necrotic area (Micrograph 19). Cases of (HN) were associated with severe endothelial destruction of hepatoportal blood vessel associated with intravascular hemolysis and dilatation of sinusoids. These severe hepatopancrease alterations were recognized and repeated in fish fromponds no.5,6,9. However, exposure to 0.33 mg UIA-N l^{-1} induced gill damage and subsequent liver tissue hypoxia associated with blood anemia as illustrated by Nasr *et al.*, [8]. Pond no. 5, 6 were located in same wind direction, held polycuture (Tilapia niloticus and Mugil) and obtained the frys from their internal hatcheries. These ponds had used fertilizers that may contribute to the increased phosphate, total solids and coliform count Ponds no. 5, 6 characterized by highest mean values of PO4-, EC,TS, TSS, NaCl%, while increased alkalinity, T. Coliform. C, TCC in pond no. 5.

The increased organic chemicals (may be phosphates) as well organic matter bearing highest value of T. Coliform and Colony counts may be contributed to some of liver severe lesions [12], where they found out liver necrosis had been shown in Nile Tlapia (O. niloticus) after exposure to sediment containing a variety of organic chemicals. Ponds no. 7, 8, 9 obtained frys from external hatcheriessource, located in same wind direction and had polyculture (Tilapia niloticus and Mugil). These ponds that held polyculture may exert different sources of excreta and wastes with increased microbial load from these excreta as manifested by increased T.Coliform. C in ponds 7,8. These Ponds had increased alkalinity mean values. Pond no.9 characterized by increased T.Coliform. C. Decreased mean values of NaCL%, EC,TS,TDS but decreased COD. This pond didn't use fertilizers and had aerator (Paddlewheel).

Due to the previously recorded values and management procedures applied in the mentioned ponds, the histological alterations noticed in liver can be attributed to the multiple concurrent environmental pollutants under filed condition. Meanwhile, fish liver was a good indicator of aquatic environmental pollution, where one of the important functions of the liver is to clean of any poisons or pollutants from the blood coming from the intestine [47]. A constant exposure to toxicants might cause damage to liver tissue [48]. Liver histopathological alterations could be expected as the liver was the main detoxification organ involved in the metabolism and excretion of xenobiotic chemicals [6]. The vacuolation of hepatocytes in the liver might be evident after exposure to the herbicide [13]. A prominent feature of chronic inflammatory responses was the presence of melanin-or other pigment-containing macrophages. These cells form discrete aggregates (MMCs) with sequestered particulate material. These were detected in the hepatic parenchyma of control and exposed fish (in the laboratory toxicity test) and in fish from contaminated ponds (in the field study) as illustrated, [49]. The hepatic lesions in fish were characterized by cloudy swelling of hepatocytes, lipoid vacuoles, pyknotic nuclei and focal necrosis which grew with increasing concentration of ammonia. Although some of the changes were reversible, the rest were less pronounced after a recovery period; a period of 10 days was not long enough for complete recovery from ammonia stress exposure [50].

Spleen: The microscopic examination of the spleen revealed lymphoid depletion (LD) associated with congestion of splenic sinusoids (Micrograph 20). The lesion associated with activation of Melanomacrophages center (MMC) (Micrograph 21). The necrosis also involved the splenic ellipsoids (Micrograph 23).Individual cases encysted metacercaria in splenic tissue was noticed (Micrograph 24). Advanced lesions were recognized in spleen as LD and necrosis in fish from ponds 5,6,9. Pond 9, showed hepatopancreatic changes, From the previous records, severe hepatic and splenic lesionscan be postulated that these ponds might be exposed to chemical pollutants, bacteria, fungi or parasites [11].Moreover, various hazards were associated with waste-fed aquaculture: excreta-related pathogens (bacteria, helminthes, protozoans and viruses), skin irritants, vectors that transmit pathogens and toxic chemicals. Fish passively accumulate microbial contaminants on their surfaces but they rarely penetrate into edible fish flesh or muscle except for trematodes (parasitic tissue flukes) [52].

In ponds 7,8,9 the absence of fertilizers and use of aerators may contribute to the low values of TS,TSS and EC, as previously concluded by many researchers [29, 24]. These decreased solids lowered the impact of turbid water and the resulted less severe histopathological lesions [11]

Brain: Brain edema was the main characteristics (HPA) in most examined cases of Tilapia. The lesion characterized by vacuolation of brain tissue (Micrograph 25).In severe cases the brain showing encephalitis that characterized microscopically by congestion of cerebral blood capillaries (Micrograph 26) associated with neuronal degeneration (Micrograph 27) and severe necrosis and demylination of brain tissue (Micrograph 28) with extravasations of free RBCs (Micrograph 29), in addition to aggregation of EGCs in brain tissue (Micrograph 30). The severe lesions were recognized in fish from ponds 5, 6, 9. These ponds were located in same wind direction, obtained fry from internal hatchery and had polyculture (Tilapia niloticus and Mugil) except pond 9 had frys from external hatchery. Pond 5 had maximum mean values of temperature, PO4+ (spring and summer) TS,TSS (summer and autumn), while pond 6 had maximum values of Chlorides, TDS, EC, NaCL% (summer and autumn).

Further study may be suggested to recognize the singular or concurrent effects of different environmental components on fish performance under seasonal climate change.

CONCLUSION

Absence of aerators in 8/10 of ponds decreased DO with consequent higher TAN values recorded during hot period with accumulation of the dissolved salts in water.. Many of water quality parameters were involved in decreased FBW, as increased pH, ammonia (mainly unionized form), increased EC, TS, TSS, TDS mainly during summer and autumn.pathological lesions of fish gill as well as liver, spleen and brain responses to water quality change were obvious.

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