Mortality rate and histopathological changes of *Oreochromis niloticus* gills experimentally exposed to *Escherichia coli*

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Abstract: Fish of *Oreochromis niloticus* were treated with different concentrations of Enterotoxigenic *Escherichia coli (ETEC) as* (10^3-10^5, 10^6-10^7 and 10^9-10^{10}/ml water). Samples of water and fish were collected at 1st, 3rd, 5th, 7th and 9th day respectively. Incidence of mortality rate for fish under experiment, the highest concentration at phase 3 which was clear as 100%, 83.3%, 25% and 00% in phases 3, 2, 1 and control respectively**.** Incidence of ETEC isolated from gills of fish under-experiment, were isolated from gills of fish as highest concentration in phase 3as 50, 75, 75, 75, and 75%. Its isolation was highest in the 9th day of each phase as 75%. Incidence of re-isolation of ETEC from water used of fish under-experiment, were re-isolated from each phase, as well high concentration especially in phase3 as 25, 50, 75, 100, and 100% respectively. The re-isolation was clear at 9th day of each phase. Fish gills were exposed to three successive concentrations. Histopathogical changes included hyperplasia of epithelial cells, degeneration and necrosis in addition to telangiectasis in the first concentration (10^3- 10^5/ml water of *E. coli*). Exposure to the second concentration (10^6- 10^7/ml water of *E. coli*) caused complete fusion of secondary lamellae, edema, necrosis and hemorrhage. After treatment with the last concentration (10^9- 10^{10}/ml water of *E. coli*), all fish specimens died in the 6th day after demonstration of severe hemorrhage and hyperplasia of epithelial cells. The results revealed the hazardous effects of water polluted with *E. coli* on the health status of *O. niloticus* and human consumption.

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Key words: *Oreochromis niloticus*, Mortality rate, gills, histopathology, *Escherichia coli.*

Introduction

Nile tilapia *Oreochromis niloticus* belongs to the family of Cichlidae and is known to the ancient Egyptian for more than five thousand years ago. It was common in natural fisheries in Africa, such as Nile River and freshwater lakes. The original home of tilapia is the content of Africa. Because of its importance, it has been introduced and spread in the world where there is warm water appropriate for their growth and reproduction especially in Southeast Asian countries (Dey *et al.,* 2000).

Most tilapia species are suitable for aquaculture. The growth rate varies depending on the nature of the food. It feed mainly on phytoplankton, algae, aquatic plants, remaining of decaying organic material and insect larvae (Tharwat, 2013).

Fish are susceptible to wide variety of bacterial pathogens especially when the fishes are physiologically unbalanced or nutritionally deficient, or subjected to stressors, i.e. poor water quality and overstocking. Infectious diseases are the main cause of economic losses in aquaculture industry which is negatively impacted by various pathogenic organisms such as *Escherichia coli* (Plumb, 1997).

Escherichia coli is G-negative rods within the family Enterobacteriaceae, and represents a part of the normal micro flora of the intestinal tract of human and warm- blooded animals. Due to their high prevalence in the gut, *E. coli* is used as the preferable indicator to detect and measure fecal contaminate in the assessment of food and water safety. Pathogenic *E. coli* strains are distinguished from other *E. coli* by their ability to cause serious illness as a result of their genetic elements for toxin production, adhesion and invasion of host cells, interference with cell metabolism and tissue destruction (Borgatta *et al.*, 2012). Historically, cultured fish were not considered important factor of human pathogens. This situation is changing due to increasing awareness by health care providers of pathogens in aquatic species that may results in human illness (Greenlees *et al.,* 1998).

Histopathology could be used as a biomarker for the effect of various pollutants on different organisms such as fish (John and Prakash, 2003). On the other hand, gills constitute the boundary tissue of the fish, continuously hydrated, nonkeratinized and first receiver of water born toxicants. The gill epithelium is sensitive to environmental variations, responding to the

functional need by the lesions in the tissue structure (Pawert *et al*., 1998). Tilapia caught from streams that receive untreated domestic waste water present gills with hyperplasia and detachment of the epithelium from the filaments and lamellae, fusion of lamellae, hypertrophy and hyperplasia of chloride cells, hemorrhage with epithelium, rupture and aneurysm (Lupi, 2006). Fontainhas- Fernandes *et al.* (2008) verified that adult tilapia exposed to waste water from sewage treatment plant had edema with detachment of lamellar and filament epithelium and lamellar fusion, cell proliferation with thickening of gill filament.

Microbiology of different tissues and gut contents from six different fish species cultured in a sewage-fed pond was studied. The total bacterial count and pathogenic flora (salmonella, coliform and faecal *Strept.*) of the raw sewage, oxidation pond and culture pond were also analyzed. The bacterial load was higher in the gut contents than in skin, gills and muscle (Balasubramanian *et al*., 1992). The treated effluent conformed to WHO guidelines were used for rearing two types of local fish (tilapia and gray mullet). The produced fish were subjected to an extensive monitoring program. Bacteriological examination revealed that in all samples the fish muscles were free of bacterial contaminants. Nevertheless, low levels of *E. coli* and *Aeromonas hydrophila*, were isolated from the surface of the fish (Easa *et al*., 1995).

Bacteriological examination of the various organs (liver, kidney, intestine and inner muscle) of four freshwater fish species belonging to the family Cyprininae reared in experimental ponds were compared to those reared in conventional pond. A total of 16 bacterial species were recovered from the water samples and the various organs of the fish. The intestines of all the fish species harbored the most number of different bacterial species. No bacteria found in the muscle of any of the fish. In general, the bacterial species isolated from the intestine were also found in the water samples from the ponds (Apun *et al*., 1999). In a study of salmon by placing it in three cages: First cage (River Water Only (RWO) trout),Second cage, (River and Waste Water (RWW) trout), Third cage,(Tab Water Reference(TWR) trout). After 66 days the mortality rate in group RWW was 87 %, in group RWO 29% and in group TWR 0 %. Only in RWW trout were ulcerations of the upper and lower jaw found, together with significantly more severe histological liver alterations and a higher lactate dehydrogenase (LDH) enzyme level in the blood (Escher *et al.,* 1999).

The aim of this work: Knowledge of the health status of fish by examining the pathological changes in the tissues of the organs directly exposed to pollutants such as gills and their impact on the consumer in order to avoid the damage to public health and achieve sustainable development.

Material and Methods

Experiment preparation of ETEC fish exposed:

- *O. niloticus* fish were bought from a fish farm and transferred to water aquaria for acclimatization. Weights of tilapia fish were (200-250 gm.).
- Tap water was used in the experimental aquaria. The physicochemical characters of tap water were measured. The results revealed that tap water was free of bacterial pollution, nitrite and ammonia (Table 1).
- Gotten isolation of *ETEC* from authorized laboratory and prepared the concentration for experiment, and exposed the fish to different concentrations (McFarland, 2009).
- All sinks were filled with de-chlorinated water at room temperature. *O. niloticus* were exposed to control and three consecutive doses of *ETEC*.
- Sampled of fish and water on control and consecutive periods were collected according to plan at (1^{st}, 3^{rd}, 5^{th}, 7^{th}, and 9^{th} days) of experiment.
- *ETEC* were isolated and classified from the gills of under experiment fish and water (Cline. Lab., 2011; CDC, 2013).

Table 1: Physicochemical characters of tap water where fish survived in the bioassay aquaria.

Parameters	Concentrations
Temperature	25 °C
pH	7.9
Total dissolved solids	95.7 mg/L
Chemical Oxygen demand (COD)	2.36 mg/L
Na^{+}	110 mg/L
K^{+}	3.4 mg/L
Ca^{++}	16.00 mg/L
Mg^{++}	0.96 mg/L
Chloride Cl^{-}	6.7 mg/L
Bicarbonate HCO_3^{-}	190
Phosphate PO_4^{-3}	0.47
Ammonia NH_3^{-}	0.0
Nitrate NO_3^{-}	1.1 mg/L
Nitrite NO_2^{-}	0.0
Total Bacteria Count/100ml	0.0
E. coli/100ml	0.0
Fungi	0.0

Histopathological preparation:

Nile tilapia (*O. niloticus*) fish were dissected and specimens of gills were removed, washed in saline solution and prepared for histological study. Gill specimens were fixed in 10% formalin for 48 hrs.

- After fixation, fish gills were dehydrated in ascending grades of ethanol, cleared in pure xylene then embedded in paraffin wax.

- The paraffin wax blocks were serially sectioned with microtome at 5 micrometers.
- Finally, the sections were mounted on glass slides, stained with hematoxylin and eosin (Bernet *et al*., 1999).
- The stained sections were examined and photographed with OMAX light microscope.
- with USB digital build in camera.

3. Results

Table and diagram 3 show incidence of ETEC isolated from gills of fish under-experiment, ETEC were isolated from gills of fish as highest concentration in phase 3as 50, 75, 75, 75, and 75%. Its isolation was highest in the 9th day of each phase as 75%.

Table and diagram 2: Incidence of mortality rate of fish under-experiment

Item	Control	Phase 1 10^3-10^5/ml water	Phase 2 10^6-10^7/ml water	Phase 3 10^9-10^{10}/ml water
Total No.	12	12	12	12
Died fish No.	00	3	10	12
Mortality rate	00/12=(00%)	3/12=(25%)	10/12=(83.3%)	12/12=(100%)

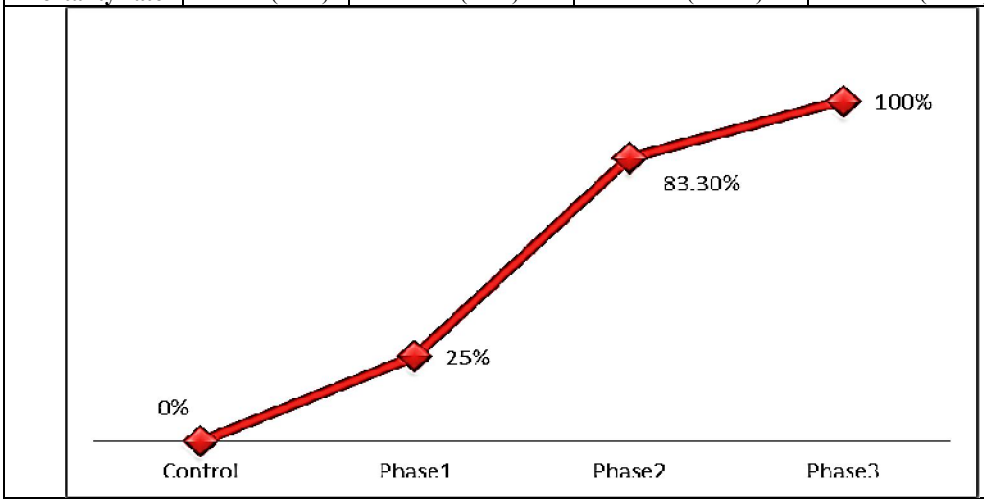


Table and diagram 2 show incidence of mortality rate of fish under experiment, the most concentration at phase 3 mortality which were clear as 100%, 83.3%, 25% and 00% in phases3, 2, 1 and control respectively**.**

Table and diagram 3: Incidence of ETEC isolated from gills of fish under-experiment

Item	Day	Control	Phase 1 10^3-10^5/ml water	Phase 2 10^6-10^7/ml water	Phase 3 10^9-10^{10}/ml water
Serial day	**1st**	00%	00%	25%	50%
	3rd	00%	25%	50%	75%
	5th	00%	50%	75%	75%
	7th	00%	75%	75%	75%
	9th	00%	75%	75%	75%

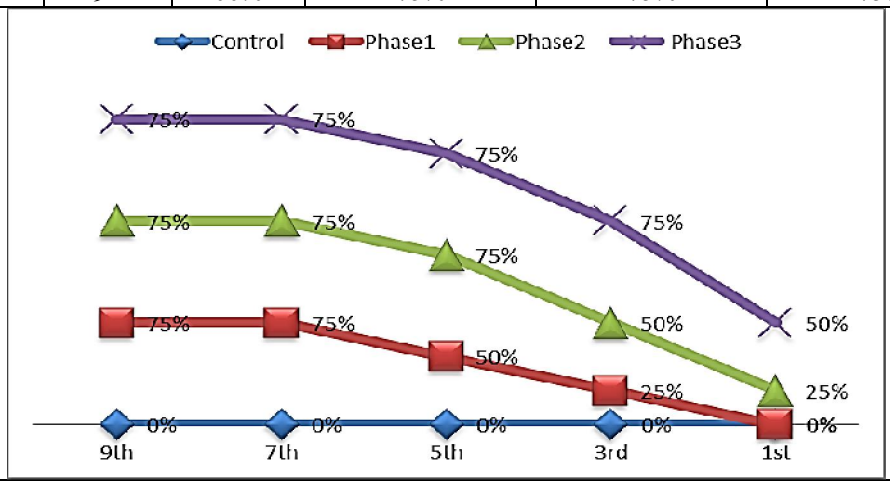


Table and diagram 4: Incidence of re-isolation of ETEC from water used of fish under-experiment

Item	Day	Control	Phase 1 10^3-10^5/ml water	Phase 2 10^6-10^7/ml water	Phase 3 10^9-10^{10}/ml water
Serial day	Before	00%	00%	00%	00%
	After 1st	00%	25%	25%	25%
	After 3rd	00%	25%	50%	50%
	After 5th	00%	50%	50%	75%
	After 7th	00%	75%	75%	100%
	After 9th	00%	75%	100%	100%

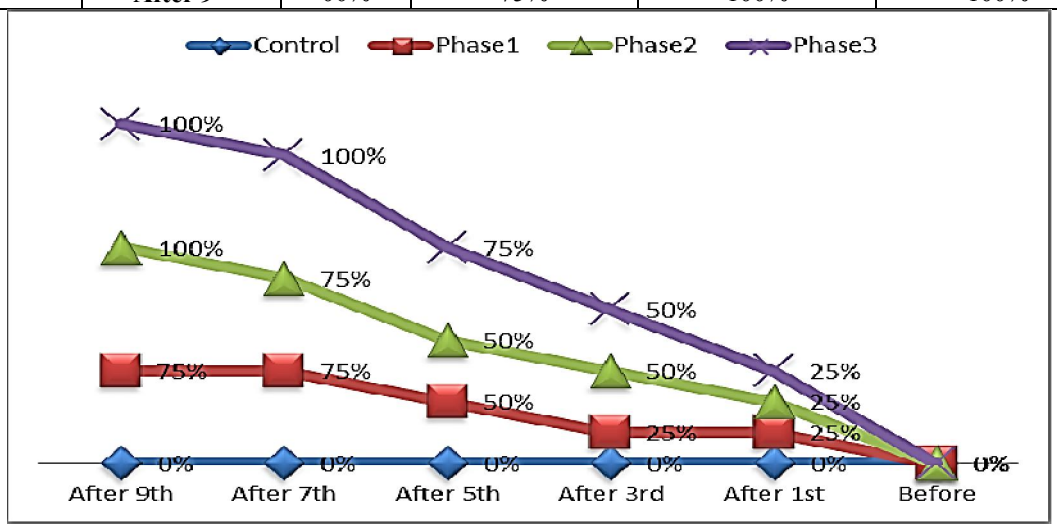


Table and diagram 4 show incidence of re-isolation of ETEC from water used of fish under-experiment, ETEC were re-isolated from each phase, were in high concentration especially in phase 3 as 25, 50, 75, 100, and 100% respectively. The re-isolation was clear after 9th day of each phase.

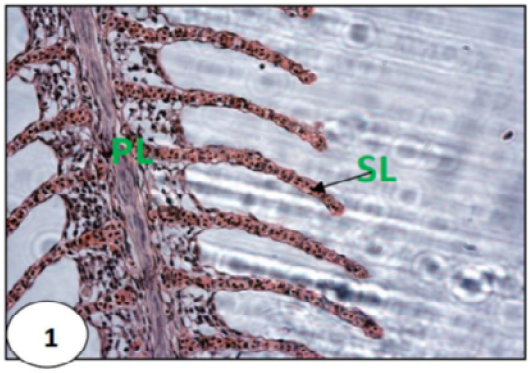
The gills of *O. niloticus* fish consists of four gill arches (similar to all teleost fish) on either side of head. They contain primary lamellae surrounded by secondary lamellae. The entire mass of primary lamellae is covered by stratified squamous epithelium. The surface of the secondary lamellae is covered with a delicate layer of a simple squamous epithelium. Inside this epithelium, there is a lamellar blood sinuses separated by the pillar cells. In the core of the primary lamellae, there is a rigid mass of cartilaginous tissue. Among the epithelial covering of the secondary lamellae, some mucous and chloride cells are located. In the control group of this experiment, the gill arches showed normal arrangement pattern except slight epithelial degeneration of primary lamellae (Fig. 1).

Pathological changes appeared in the 5th day of exposure to concentration (10^3-10^5/ml water) of *E. coli*. Microscopic observations showed necrosis of epithelial cells and fusion of secondary lamellae (Fig. 2).Hyperplasia and degeneration of epithelial cells in the 7th day of exposure (Fig. 3). In the 9th day (the last day of the experiment), sections of gills showed degeneration and necrosis of epithelial cells in addition to telangiectasis (Figs. 4&5).

After exposure to concentration (10^6-10^7/ml water) of *E. coli*, the gills section of *O. niloticus* in the 3rd day illustrated degeneration, necrosis of epithelial cells, edematous separation of primary and secondary lamellae in some filaments, complete fusion of secondary lamellae accompanied by edema and necrosis. The gill filaments lost their normal architecture (Figs.6, 7&8). Gills specimens in the 5th day of exposure showed edema, hyperplasia of epithelial cells and hemorrhage in the primary lamellae (Figs. 9&10), degeneration, necrosis, detachment of epithelial cells, curling of secondary lamellae and severe hemorrhage were demonstrated in (Figs. 11-14) in the 7th day of exposure. In the last day of exposure to concentration (10^6-10^7/ml water), (the 9th day), the gill sections showed the deleterious pathological changes including edema, necrosis, fusion of secondary lamellae and hemorrhage (Fig. 15).

After treatment with concentration (10^9-10^{10} /ml water) of *E. coli*, all fish samples died in the 6th day. The gill specimens were taken only in the 1st, 3rd and 5th day of experiment. The histo-pathological alterations were observed in the first day as hyperplasia of epithelial cell and aneurism (Fig. 16), degeneration,

necrosis, edematous separation of epithelial cells and infiltration of blood in the 3rd day of exposure (Figs. 17, 18 &19). In the 5th day (before fish spend), the fish gills showed severe hemorrhage and hyperplasia of epithelial cells (Fig. 20).



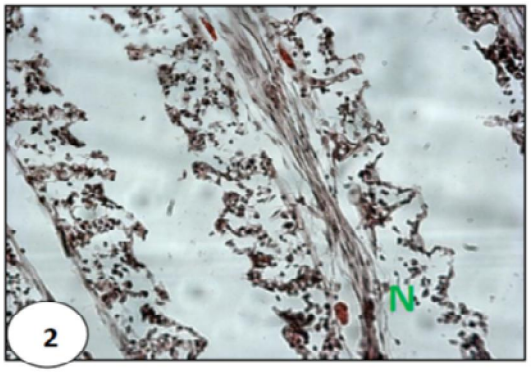
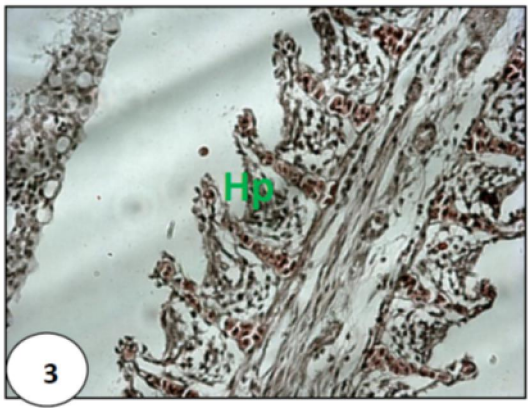


Fig. 1: normal structure of gills of *Oreochromis niloticus* from the control group, showing primary lamellae (PL) and secondary lamellae (SL) of a gill filament. X400

Fig. 2: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^3-10^5/ml water) of *E. coli* in the 5th day of exposure, showing fusion of secondary lamellae and necrosis of epithelial cells (N). X400



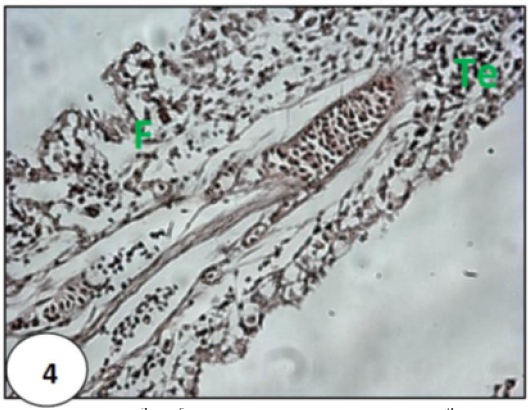
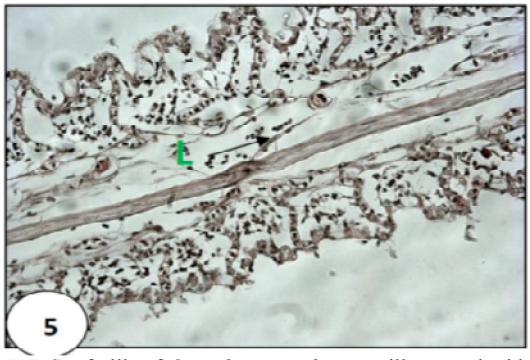


Fig. 3: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^3-10^5/ml water) of *E. coli* in the 7th day of exposure, showing degeneration and hyperplasia (Hp) of epithelial cells of secondary lamellae. X400

Fig. 4: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^3-10^5 /ml water) of *E. coli* in the 9th day of exposure, showing degeneration and complete fusion of secondary lamellae (F) and telangiectasis (Te) at the tip of the filaments. X400



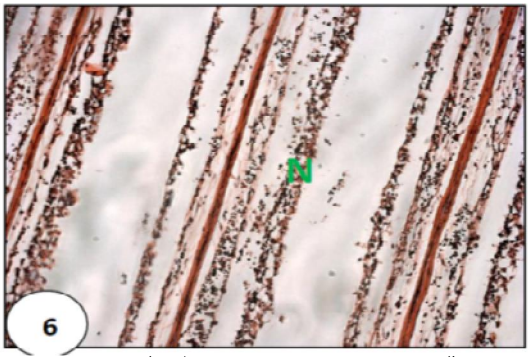
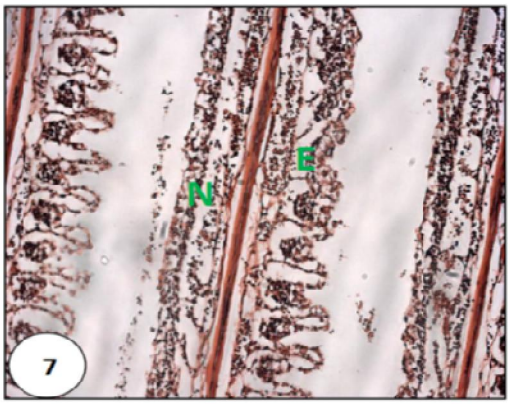


Fig. 5: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^5-10^5/ml water) of *E. coli* in the 9th day of exposure, showing infiltration of leukocytes (L) within necrotic epithelial cells. X400

Fig. 6: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7 /ml water) of *E. coli* in the 3rd day of exposure, showing complete fusion and necrosis of secondary lamellae (N). The gill filaments lost their normal archeticture. X200



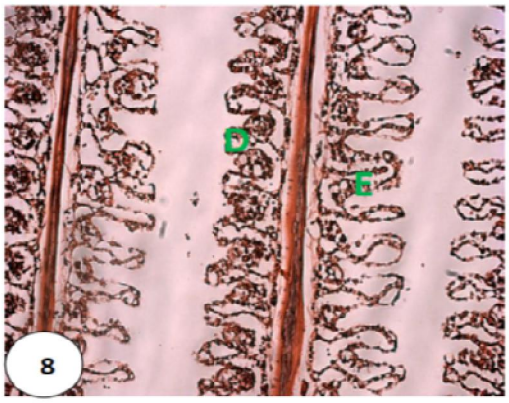
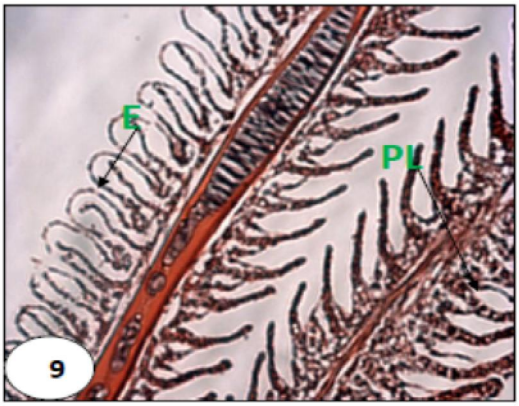


Fig. 7: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7 /ml water) of *E. coli* in the 3rd day of exposure, showing complete fusion of secondary lamellae, necrosis of epithelial cells (N), edematous separation of primary and secondary lamellae (E). X200

Fig. 8): L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7 /ml water) of *E. coli* in the 3rd day of exposure, showing sever edematous separation of primary and secondary lamellae (E) and degeneration of epithelial cells (D). X200



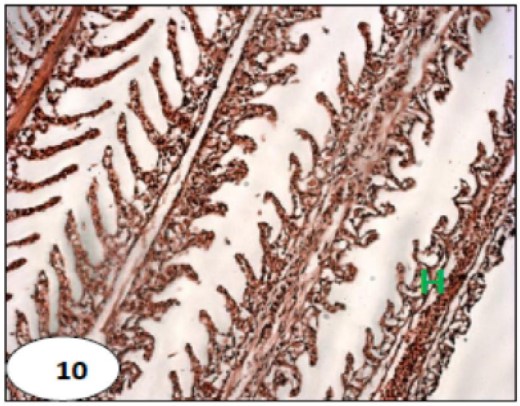
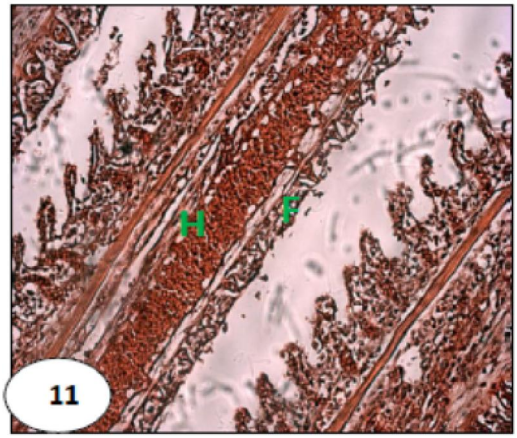


Fig. 9): L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7/ml water) of *E. coli* in the 5th day of exposure, showing sever edematous separation of primary and secondary lamellae (E) and proliferation of epithelial cells in primary lamellae (PL). X200

Fig. 10): L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7 /ml water) of *E. coli* in the 5th day of exposure, showing hemorrhage (He) in the axon of the filaments. X200



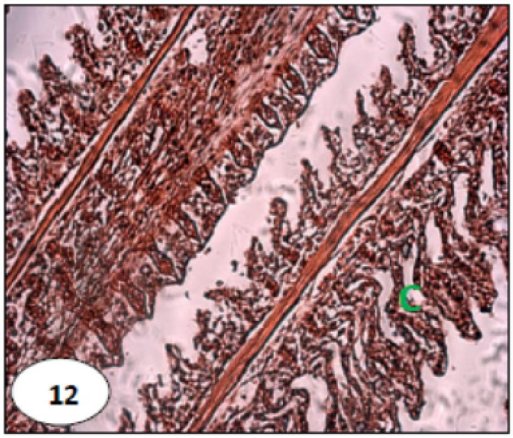
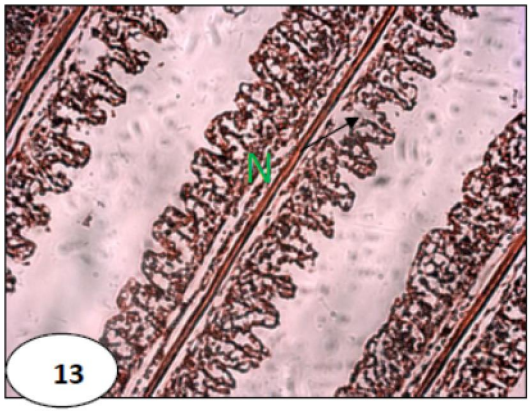


Fig. 11): L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7 /ml water) of *E. coli* in the 7th day of exposure, showing severe hemorrhage (He) and fusion of secondary lamellae (F). X200

Fig. 12: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^6-10^7 /ml water) of *E. coli* in the 7th day of exposure, showing severe hemorrhage and fusion of secondary lamellae in addition to curling © within the secondary lamellae. X200



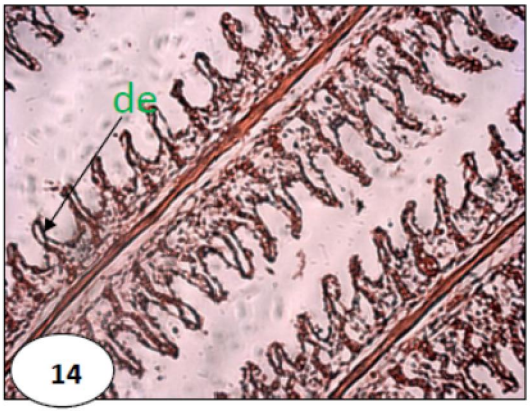


Fig. 13: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^{6}-10^{7} /ml water) of *E. coli* in the 7th day of exposure, showing degeneration and necrosis (N) of epithelial cells. X200

Fig. 14: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^{6}-10^{7} /ml water) of *E. coli* in the 7th day of exposure, showing degeneration, necrosis and detachment of epithelial cells in the secondary lamellae (de). X200

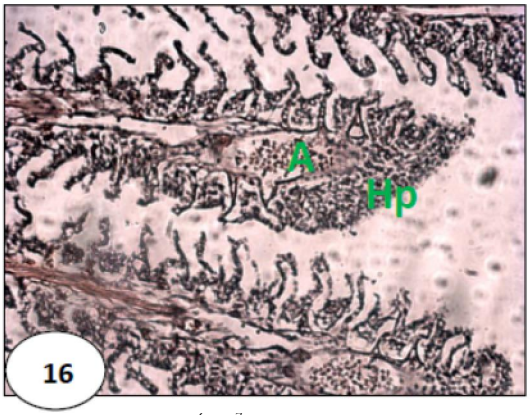
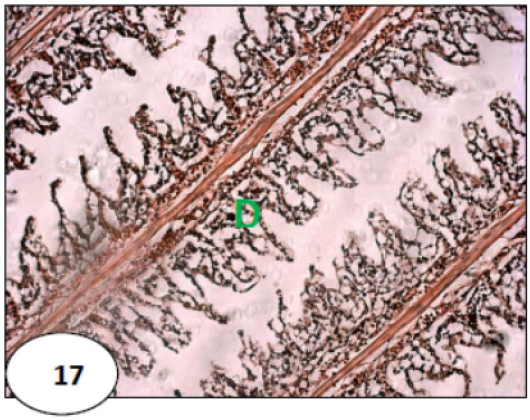


Fig. 15: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^{6}-10^{7} /ml water) of *E. coli* in the 9th day of exposure, showing complete fusion, necrosis and edema (E) of secondary lamellae and hemorrhage (He) of primary lamellae. X200

Fig. 16: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^{9}-10^{10}/ml water) of *E. coli* in the 1st day of exposure, showing dilation and congestion of blood vessel (aneurism) (A) and hyperplasia of epithelial cells at the tip of the filament (Hp). X200



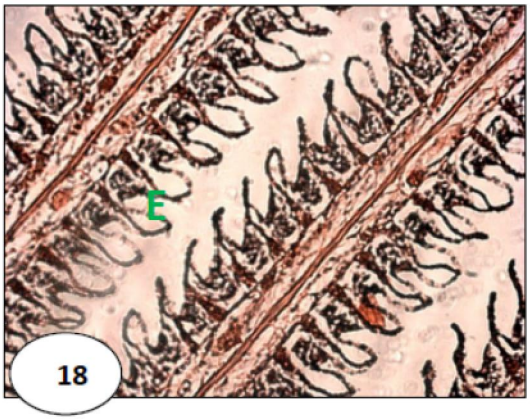
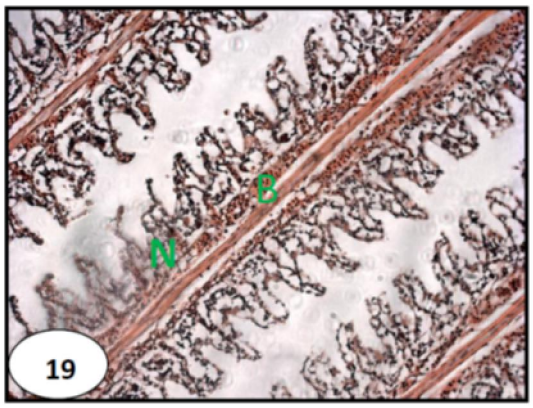


Fig. 17: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^{9}-10^{10}/ml water) of *E. coli* in the 3rd day of exposure, showing degeneration (D) and necrosis of epithelial cells. X200

Fig. 18: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^{9}-10^{10}/ml water) of *E. coli* in the 3rd day of exposure, showing edematous separation of primary and secondary lamellae (E) in addition to infiltration of blood (B) in primary lamellae. X200



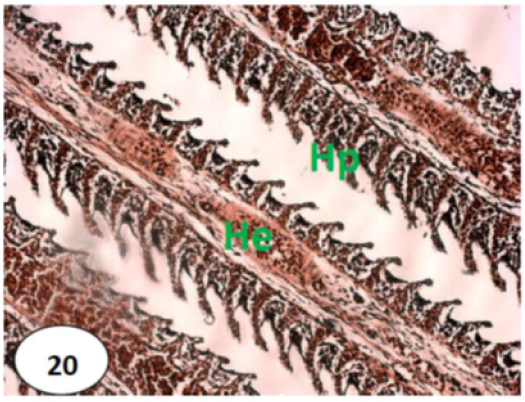


Fig. 19: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^9-10^{10} /ml water) of *E. coli* in the 3rd day of exposure, showing degeneration, necrosis (N) of epithelial cells and infiltration of blood (B) in primary lamellae. X200
Fig. 20: L.S. of gills of *Oreochromis niloticus* gills treated with concentration (10^9-10^{10}/ml water) of *E. coli* in the 5th day of exposure, showing severe hemorrhage (He) and hyperplasia (Hp) of epithelial cells. X200.

Discussion

Table and diagram 2 show incidence of mortality rate of fish under experiment, the highest concentration at phase 3 mortality which were clear as 100%, 83.3%, 25% and 00% in phases 3, 2, 1 and control respectively**.**

Table and diagram 3 show incidence of ETEC isolated from gills of fish under-experiment, ETEC were isolated from gills of fish as highest concentration in phase 3as 50, 75, 75, 75, and 75%. Its isolation was highest in the 9th day of each phase as 75%.

Table and diagram 4 show incidence of re-isolation of ETEC from water used of fish under-experiment, ETEC were re-isolated from each phase, were in high concentration especially in phase3 as 25, 50, 75, 100, and 100% respectively.

The gill epithelium of the fish is the major site of gas exchange, acid base balance, ionic regulation and excretion of nitrogenous wastes. Consequently, the epithelium that covers the gill filaments and lamellae provides a distinct boundary between a fish's external environment and extracellular fluids and also plays a critical role in the physiological function of the fish gills. The presence of mucus filled cavity (edema) observed in the gill filaments of *O niloticus* in the present experiment may be considered as an ion trap, in a way to concentrate free elements from surrounding water between the neighboring lamellae (Thopon *et al.,* 2003).

El Sayyed *et al.* (2010) observed massive alteration of histological and scanning ultrastructures of infected gills with pathogenic bacteria including *E. coli* as degeneration of the lamellae and cellular hypertrophy structure lead to decrease in the respiratory capacity between the lamellae, impairs the diffusion of oxygen across the swollen condition of the epithelium and decrease in free gas exchange which in turn increase the mortality of fish.

Winkaler *et al.* (2001) and Fontainhas- 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, hyper secretion and proliferation of mucosal cells. The presence of telangiectasis in the gills of *O. niloticus* in 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 (Debora *et al*., 2012).

The histological changes in the gills of *O. nilticus* in this study may be adaptation to prevent pollutant entry through gill surface (Fernandes and Mazon, 2003). Likewise, Camargo and Martinez (2007) observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *P. lineatus* being caged in Brazilian Cambe stream being polluted by the industrial, domestic and agricultural wastes. Triebskorn *et al*. (2008) reported epithelial lifting, proliferation, necrosis and hyperplasia of mucous cells in gills of *C. nasus* and *L. cephalus* from River Mures in Western Romania which was polluted by heavy metals, faecal coliforms and streptococci bacteria.

Consequently, injury to the gill epithelium is a common response observed in fish exposed to variety of contaminants. Arellan *et al*. (2000) and Jabeen and Chaudhry (2013) reported the fusion of adjacent lamellae after exposure to heavy metals, such as cadmium and copper which agreed well with the present findings. The lifting of the lamellar epithelium and lamellar fusion could be protective as it diminished the extent of vulnerable gill surface area (Van Hardeen *et al*., 2004).

For respiration, the gills are highly vascularized, with a large number of blood capillaries, therefore, they may provide good entry site for bacteria to become

easily disseminated to the entire body of the fish (Ling *et al*., 2001). This may indicate that, bacteria first adhere to gill mucous and thereafter invade the branches vasculature leading to colonization of the internal organs (Torroba *et al*., 1993).

Conclusion

The present results of the experiment revealed that the gills of *O. niloticus* was highly susceptible to *E. coli* infection inducing pathological alterations and finally caused mortality in the highest concentration. It is recommended to check the validity of the fish stocked in fish farms on the sewage or that live in the waters of the seas or rivers contaminated with sewage for human consumption to prevent the use of sanitation on farms.

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