ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Impact of *Escherichia coli* toxins on blood chemistry and intestine of *Oreochromis niloticus*

[1]Amal M. Yacoub*, [2]Sherifa Mostafa M. Sabra, and [3]Mona Khaled D. Al-Kourashi.

[1, 2 &3]Biology Dept., Science College, Taif University, KSA.
[1]Pollution Lab. / National Institute of Oceanography and Fisheries.
[2]Department of Microbiology / Animal Health Research Institute, Dokki, Giza, Egypt.

ABSTRACT

Fish of *Oreochromis niloticus* (Linnaeus, 1758) were infected with different concentrations of Enterotoxigenic *Escherichia coli (ETEC)* as (10^3-10^5, 10^6-10^7 and 10^9-10^{10} CFU /ml water). The study had conducted to detect experimentally the impact of *E. coli* toxins on *O. niloticus* fish throughout the blood chemistry and histopathological changes of intestine. The values of glucose, cholesterol and triglycerides ranged between (346.42 mg/dl- 1.09 mg/dl), (279.45 mg/dl- 87.32 mg/dl) and (413.39 mg/dl- 78.84 mg/dl) respectively. The intestine of *O. niloticus* infected with *E. coli* showed degeneration of mucosa and submucosa, necrosis of villi, hyperplasia of goblet cells, infiltration of WBCs, hemorrhage, fragmentation and necrosis of muscular layer beside degeneration of serosa. The present investigation revealed that exposure of *O. niloticus* to *E. coli* caused disorders in metabolism and induced pathological changes in intestine which impede absorption of food stuff and finally caused mortality in the highest concentrations. It is recommended to prevent fishing in seas or rivers polluted with sewage or usage of sanitation in farms.

Keywords: experimental study, *Oreochromis niloticus*, *Escherichia coli*, blood chemistry, intestine.

https://doi.org/10.33887/rjpbcs/2019.10.4.14

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Tilapia is the second most farmed fish worldwide, and its production has multiplied over the past decade because of its suitability for aquaculture, good marketability and stable prices (Wang and Lu, 2016). 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 micro-organisms (MOs) such as *Escherichia coli* (*E. 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 by their ability to cause serious illness as a result of their genetic elements for toxins production, adhesion and invasion of the 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). Microbiology of different tissues and gut contents from six different fish spp. cultured in a sewage-fed pond, the total bacterial count and pathogenic flora coliform 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).

Bacteriological examination of the various organs (liver, kidney, intestine and inner muscle) of four freshwater fish spp. belonging to the family Cyprinidae reared in experimental ponds were compared to those reared in conventional pond. A total of 16 bacterial spp. were recovered from the water samples and the various organs of the fish. The intestines of all the fish spp. harbored the most number of different bacterial species. No bacteria were 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).

The fish is a good source of proteins for human. Untreated sewage water contains intestinal pathogens as *E. coli*. Some fish breeders use untreated sewage water for growing fish. The microbial toxins produced bad fish meat quality and may transfer pathogens to human. So this research had conducted to detect experimentally impact of *E. coli* toxins on *Oreochromis niloticus* fish understudy throughout blood chemistry including glucose, triglyceride and cholesterol and histopathogical changes of intestine to confirm the hazardous effect of using sewage water for fish growth and breeding.

MATERIAL AND METHODS

- *Oreochromis niloticus* fish were bought from a fish farm then 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 (Enterotoxigenic *Escherichia coli*) from authorized laboratory and prepared the concentration for experiment, and exposed the fish to different concentrations (Mc Farland, 2009).
- All sinks were filled with de-chlorinated water at room temperature. *Oreochromis niloticus* fish were exposed to control and three consecutive doses of ETEC (ETEC concentrations were 10^3-10^5/ml water, 10^6 -10^7/ml water and 10^9-10^{10}/ml water).
- Samples of fish and water of control and consecutive doses were collected according to plan at (1st, 3rd, 5th, 7th and 9th days) of experiment.
- *ETEC* were isolated and classified from the blood, intestine of under experiment fish and water.

ISSN: 0975-8585

Table 1: Physicochemical characters and microbial analysis 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
Ca^{++}	16.00
Mg^{++}	0.96
Chloride Cl^{-}	6.7
Ammonia NH_3	0.0
Nitrate NO_3	1.1 mg/L
Nitrite NO_2	0.0
Total coliform Bacteria /100ml	0.0
E. coli/100ml	0.0
Fungi	0.0
Dissolved O^2	2.36 mg/L
Na^{+}	110
K^{+}	3.40
HCo_3^{-}	190
PO_4	0.47

Physiological methods:

Blood samples were taken from the caudal vein of the fish using a heparinized syringe and collected into small sterilized plastic tubes. Blood samples were left to coagulate for 15-20 min. at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate serum. Samples were stored in polyethylene Eppendorff test tubes at -20°C until serum analysis. Serum samples were used to estimate, calorimetrically, Glucose, Cholesterol and Triglyceride using Stanbio kit as described by (Reitman and Frankel, 1957), (Trinder, 1959) and (Dumas and Biggs,1972).

Histological methods:

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

- After fixation, specimens 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.

RESULTS

The incidence of re-isolation of ETEC during the experiments phases were shown in (Table 2). ETEC were in 00% from control in water, intestine and blood. At 1st phase ETEC re-isolation from water was (25, 25, 50, 75 and 75%) and from intestine (25, 25, 25, 25 and 25%) at 1st, 3rd, 5th, 7th and 9th days respectively and from blood as zero at 1st, 3rd, 5th, 7th and 9th in all phases of experiment.

At 2nd phase the ETEC re-isolation from water as (25, 50, 50, 75 and 100%) and form intestine (75, 75, 75, 100 and 100%) at 1st, 3rd, 5th, 7th and 9th days respectively.

ISSN: 0975-8585

At 3rd phase, the fish did not continue (all the fish died) after the 5th day of exposure. The ETEC re-isolation from water was (25, 50 and 75%) and from intestine (100, 100 and 100%) at 1st, 3rd and 5th days respectively.

Table 2: Incidence of re-isolation of ETEC during the experiment phases

Days	ETEC *Conc.	10^3-10^5*CFU/ml 1st phase					10^6-10^7*CFU/ml 2nd phase					10^9-10^{10}*CFU/ml 3rd phase		
	Control	1st	3rd	5th	7th	9th	1st	3rd	5th	7th	9th	1st	3rd	5th
Water	00%	25 %	25%	50%	75%	75%	25%	50%	50%	75%	100%	25%	50%	75%
Intestine	00%	25 %	25%	25%	25%	25%	75%	75%	75%	100 %	100%	100%	100%	100%
Blood	00%	00 %	00%	00%	00%	00%	00%	00%	00%	00%	00%	00%	00%	00%

**Conc.: Concentration, *CFU: Colony forming unit*

Glucose concentration:

The maximum value of Glucose in concentration (10^3 - 10^5 CFU /ml) was 118.28 mg/dl in the 1st day and the minimum value was 4.85 mg/dl in the 7th day. The mean ± standard deviation was 50.05±43.92.

The maximum value of Glucose in concentration (10^6- 10^7 CFU / ml) was 346.42 mg/dl in the 1st day and the minimum value was 1.09 mg/dl in the 3rd day. The mean ± standard deviation was 89.87±149.11.

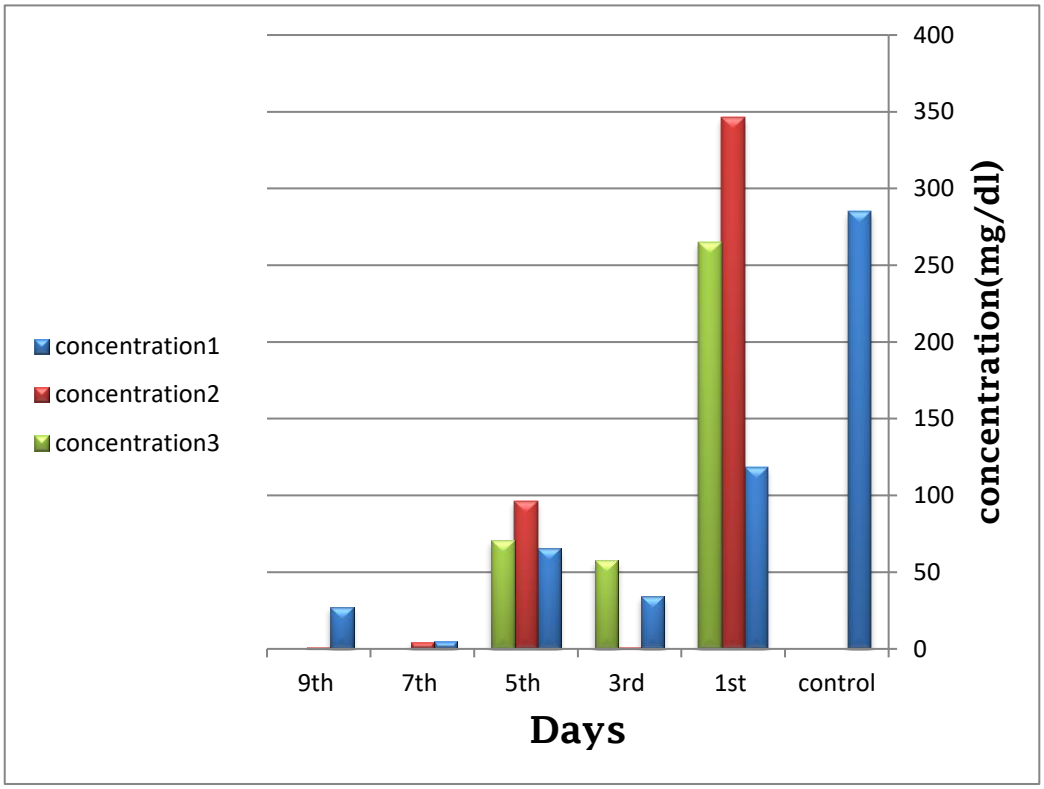
The maximum value of Glucose in concentration (10^9 – 10^{10} CFU /ml) was 265.02 mg/dl in the 1st day and the minimum value was 57.63 mg/dl in the 3rd day. The mean ± standard deviation was 131.05±116.19.

The values of Glucose concentration in the experiment ranged from the highest value 346.42 mg/dl in the 1st day in concentration (10^6- 10^7 CFU /ml) and the lowest value 1.09 mg/dl in the 3rd day in the same concentration. The control was 285.14 mg/dl as illustrated in (Table and diaphragm 3).

(Table and diaphragm 3): showing Glucose concentration (mg/dl) in blood of *Oreochromis niloticus* treated with different concentration of ETEC.

Days/ concentration	1	3	5	7	9	Mean ± STD
10^3-10^5/ml	118.28	34.02	65.76	4.85	27.38	50.05±43.92
10^6 -10^7/ml	346.42	1.09	96.43	4.26	1.15	89.87±149.11
10^9-10^{10}/ml	265.02	57.63	70.50	-	-	131.05±116.19
Mean ± STD	243.24±115.61	30.91±28.39	77.56±16.51	4.55±0.41	14.26±18.54	/

ISSN: 0975-8585



mg= milligram **dl=deciliter**

Cholesterol concentration:

The maximum value of Cholesterol in concentration (10^3 - 10^5 CFU / ml) was 269.13 mg/dl in the 7^{th} day and the minimum value was 125.5 mg/dl in the 9^{th} day. The mean ± standard deviation was 192.52±66.11.

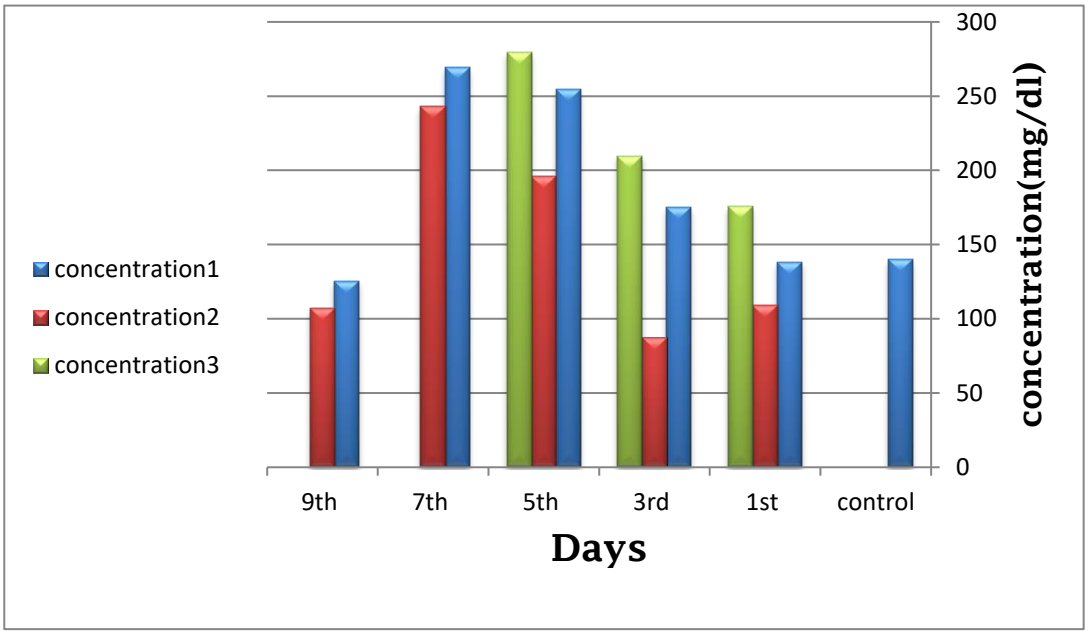
The maximum value of Cholesterol in concentration (10^6- 10^7 CFU / ml) was 242.88 mg/dl in the 7^{th} day and the minimum value was 87.32 mg/dl in the 3^{rd} day. The mean ± standard deviation was 148.40±67.37.

The maximum value of Cholesterol in concentration (10^9 – 10^{10} CFU /ml) was 279.45 mg/dl in the 5^{th} day and the minimum value was 175.54 mg/dl in the 1^{st} day. The mean ± standard deviation was 221.53±52.97.The values of Cholesterol concentration in the experiment ranged from the highest value 279.45 mg/dl in the 5^{th} day in concentration (10^9- 10^{10} CFU /ml) and the lowest value 87.32 mg/dl in the 3^{rd} day in concentration (10^6- 10^7 CFU / ml). The control was 140.05 mg/dl as illustrated in (Table and diaphragm 4).

ISSN: 0975-8585

(Table and diaphragm 4): showing Cholesterol concentration (mg/dl) in blood of *Oreochromis niloticus* treated with different concentration of ETEC

Days/ concentration	1	3	5	7	9	Mean ± STD
10^3-10^5/ml	138.29	174.93	254.77	269.13	125.5	192.52±66.11
10^6 -10^7/ml	108.91	87.32	195.73	242.88	107.19	148.40±67.37
10^9-10^{10}/ml	175.54	209.60	279.45	-	-	221.53±52.97
Mean ± STD	140.91±33.3	157.2±63.02	243.3±43.01	256±18.5	116.34±12.9	/



mg= milligram **dl=deciliter**

Triglyceride in concentration:

The maximum value of Triglyceride in concentration (10^3 - 10^5 CFU / ml) was 192.12 mg/dl in the 7th day and the minimum value was 83.06 mg/dl in the 1st day. The mean ± standard deviation was 100.83±52.01.

The maximum value of Triglyceride in concentration (10^6- 10^7 CFU / ml) was 172.00 mg/dl in the 1st day and the minimum value was 78.84 mg/dl in the 5th day. The mean ± standard deviation was 129.56±36.60.

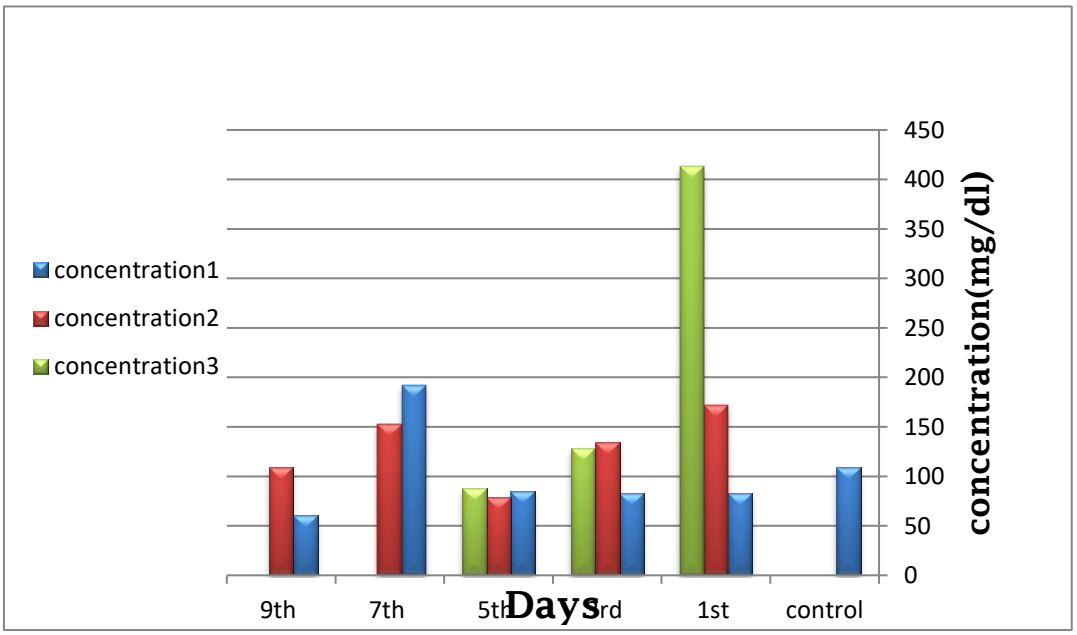
The maximum value of Triglyceride in concentration (10^9 – 10^{10} CFU /ml) was 413.39 mg/dl in the 1st day and the minimum value was 87.46 mg/dl in the 5th day. The mean ± standard deviation was 209.70±177.57.

The values of Triglyceride concentration in the experiment ranged from the highest value 413.39 mg/dl in the 1st day in concentration (10^9- 10^{10} CFU /ml)) and the lowest value 78.84 mg/dl in the 5th day in concentration (10^6- 10^7 CFU / ml). The control was 109.03mg/dl as illustrated in (Table and diaphragm 5).

ISSN: 0975-8585

(Table and diaphragm 5): showing Triglyceride concentration (mg/dl) in blood of *Oreochromis niloticus* treated with different concentration of ETEC.

Days/ concentration	1	3	5	7	9	Mean ± STD
10^3-10^5/ml	83.06	83.34	85.01	192.12	60.62	100.83±52.01
10^6 -10^7/ml	172.00	134.10	78.84	153.06	109.26	129.56±36.60
10^9-10^{10}/ml	413.39	128.26	87.46	-	-	209.70±177.57
Mean ± STD	228.8±180.9	115.2±27.7	83.7±4.4	172.5±27.6	84.9±34.3	



Histopathological changes of intestine of *Oreochromis niloticus* experimentally treated with *Escherichia coli:*

The intestine of most fish is a simple tube which does not increase in diameter to form a colon posteriorly. It may be straight, sigmoid or coiled, depending on the shape of the abdominal cavity. It has a simple, mucoid, columnar epithelium, overlying a submucosa often with abundant eosinophilic granule cells and limited by a dense muscularis mucosa and fibroelastic layer (Fig.1).

The intestine of *Oreochromis niloticus* infected with concentration (10^3-10^5 CFU /ml) of *Escherichia coli* demonstrated fragmentation and rupture of intestinal mucosa in 1st day (Fig.2) and inflammatory infiltration of WBCs(Fig.3) in the 3rd day. In the 5th day, degeneration of villi (Fig.4) in addition to mucous cells scattered within the villi and hemorrhage in the mucosal layer were noticed (Fig.5 and 6). In the 7th day and 9th day, necrosis of epithelial cells of villi was common (Fig.7 and 8).

The intestine of *Oreochromis niloticus* infected with concentration (10^6-10^7 CFU /ml) of *Escherichia coli* demonstrated shortening of villi and infiltration of inflammatory cells in 3rd day (Fig.9). In the 3rd day and 7th day, necrosis of villi and epithelial cells was shown in (Fig.10 and13). In the 5th day, epithelial cells of villi (Fig. 11) and connective tissue of submucosa degenerated (Fig. 12).In the 9th day, shortening and hemorrhage within the mucosa appeared (Fig. 14).

The intestine of *Oreochromis niloticus* infected with concentration (10^9-10^{10} CFU /ml) of *Escherichia coli* demonstrated vacuolar degeneration of villi and infiltration of inflammatory cells in 1st day and 3rd day(Fig. 15 and 17).In the 1st day, all layers of intestine deteriorated with necrosis of villi. The debris of epithelial cells were scattered in the section beside fragmentation of muscular layer of submucosa and degeneration of

ISSN: 0975-8585

serosa (Fig. 16). In the 5th day, inflammatory cells infiltrated within the villi with thickening and necrosis of muscular layer of sub mucosa (Fig. 18).

***(Fig. 1): Normal structure of intestine of *Oreochromis niloticus* fish from control group, showing mucosa, submucosa and serosa. X100**

***(Fig. 2): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 1st day of exposure, showing fragmentation and rupture (F) of intestinal mucosa. X400**

***(Fig. 3): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 3rd day of exposure, showing inflammatory infiltration of WBCs (arrow). X400**

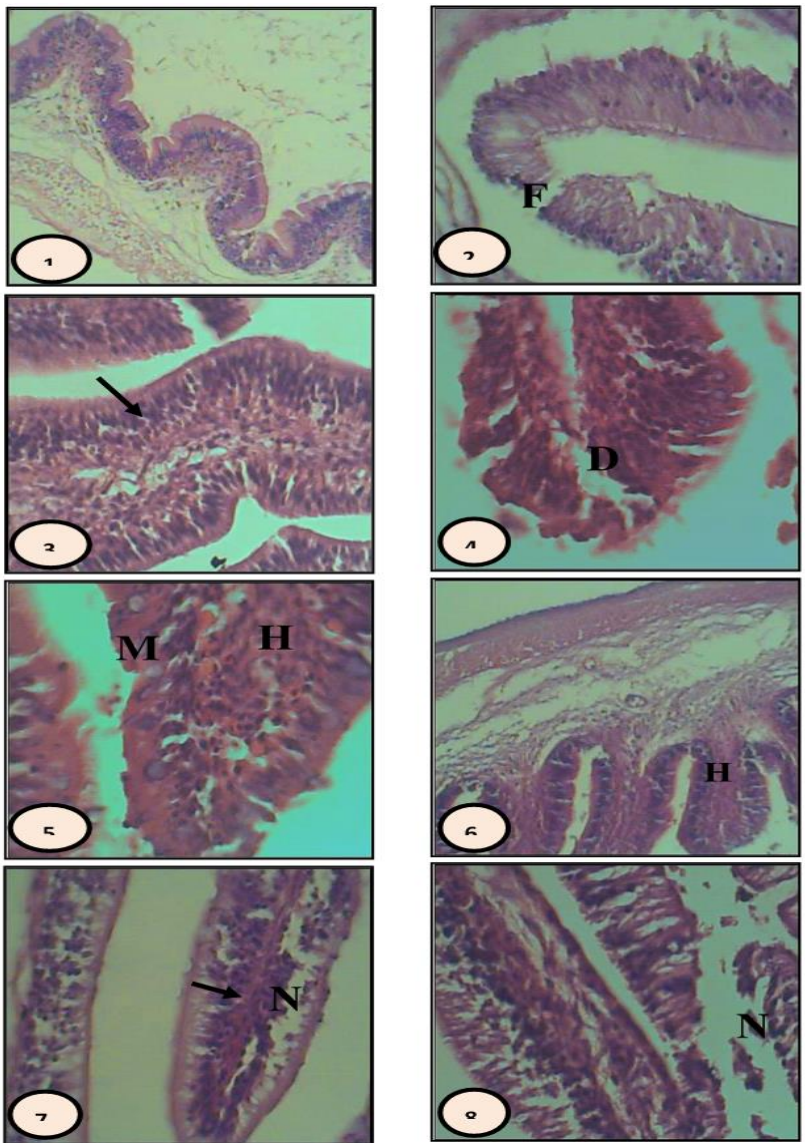
***(Fig. 4): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 5th day of exposure, showing degeneration of villi (D). X400**

***(Fig. 5): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 5th day of exposure, showing numerous mucous cells (MC) within the villi and hemorrhage in the mucosal layer (Hg). X400**

***(Fig. 6): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 5th day of exposure, reveals hemorrhage (Hg) of mucosa. X100**

***(Fig. 7): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 7th day of exposure, showing necrosis (N) and infiltration of inflammatory cells (arrows) in mucosa. X100**

***(Fig. 8): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^3_ 10^5 CFU /ml) of ETEC in the 9th day of exposure, showing necrosis of epithelial cells of villi (N).Debris of epithelial cells appeared in the picture X400**



ISSN: 0975-8585

*(Fig. 9): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^6_ 10^7 CFU /ml) of ETEC in the 3rd day of exposure, showing shortening of villi and infiltration of inflammatory cells (arrow). X400

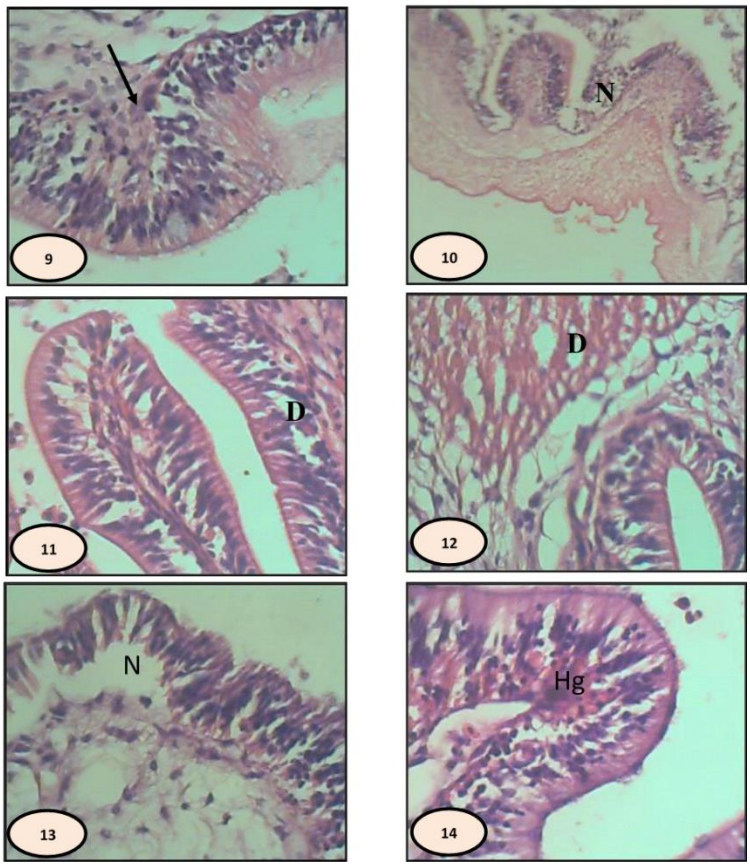
*(Fig. 10): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^6_ 10^7 CFU /ml) of ETEC in the 3rd day of exposure, showing necrosis of villi (N). The photograph demonstrated debris of epithelial cells. X100

*(Fig. 11): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^6_ 10^7 CFU /ml) of ETEC in the 5th day of exposure, showing degeneration (D) of epithelial cells of villi. X400

*(Fig. 12): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^6_ 10^7 CFU /ml) of ETEC in the 5th day of exposure, showing degeneration (D) of connective tissue of sub mucosa. X400

*(Fig. 13): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^6_ 10^7 CFU /ml) of ETEC in the 7th day of exposure, showing shortening of villi and necrosis (N) of epithelial cells. X400

*(Fig. 14): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^6_ 10^7 CFU /ml) of ETEC in the 9th day of exposure, showing shortening and hemorrhage (Hg) within the mucosa. X400



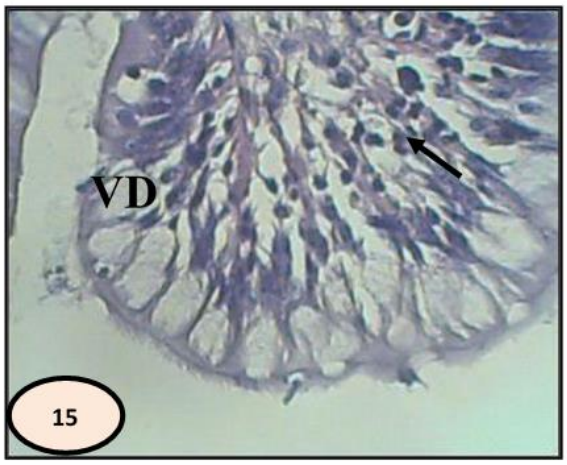
ISSN: 0975-8585

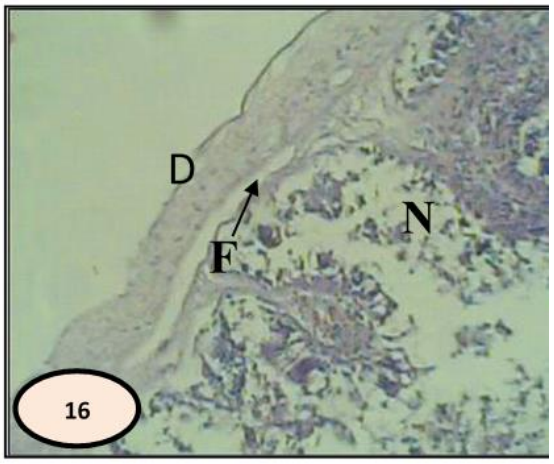
***(Fig.15): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^9_ 10^{10} CFU /ml) of ETEC in the 1st day of exposure, showing vacuolar degeneration of villi (VD) and infiltration of inflammatory cells (arrow) X400**

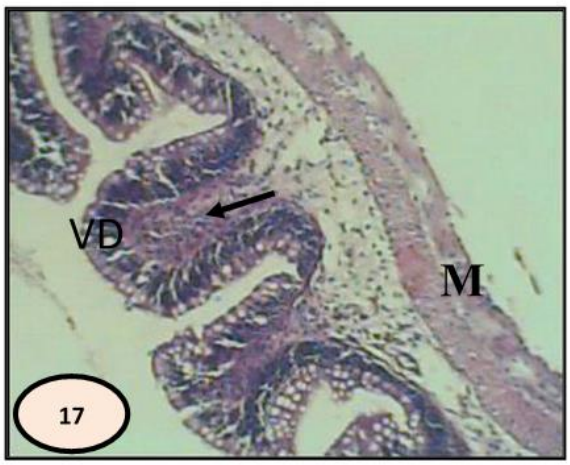
***(Fig.16): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^9_ 10^{10} CFU /ml) of ETEC in the 1st day of exposure, showing deterioration in all layers of intestine, ,necrosis of villi (N). The debris of epithelial cells are scattered in the section, fragmentation (F) of muscular layer of sub mucosa and degeneration (D) of serosa. X400**

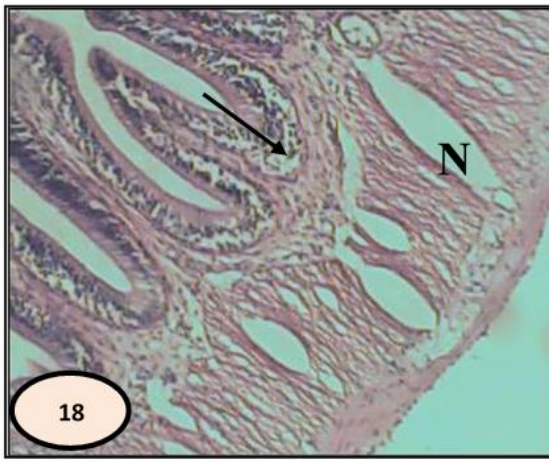
***(Fig.17): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^9_ 10^{10} CFU /ml) of ETEC in the 3rd day of exposure, showing vacuolar degeneration (VD) of epithelial cells and infiltration of inflammatory cells (arrow) in the villi and degeneration of muscular layer of sub mucosa (ML). X100**

***(Fig.18): T.S. of *Oreochromis niloticus* intestine infected with concentration (10^9_ 10^{10} CFU /ml) of ETEC in the 5th day of exposure, showing infiltration of inflammatory cells within the villi (arrow) thickening and necrosis (N) of muscular layer of sub mucosa. X100**









ISSN: 0975-8585

DISCUSSION

Fecal pollution serves as a vehicle for disease transmission including pathogenic bacteria. The type and amount of MOs found in fecal pollution is dependent on the host source (human, agricultural animal, wildlife) and the prevalence of illness in the host population. Therefore, employing fecal indicators that provide information about human and other animal contributions is critical for estimating the likelihood that pathogens are present and for directing remediation efforts (Mc Lellan *et al*., 2013).

Management of the risk of waterborne disease transmission requires knowledge about the nature of the pathogens, their potential growth, fate and transport in the water cycle, the routes of exposure to humans and the health effects that may result from this exposure in the human population, as well as the effect of potential mitigation measures (Medema, 2013). Organic sewage pollution influences benthic diversity in coastal waters by supporting communities of opportunistic characteristics. Advocate inclusion of community traits and compatible analytical tools (statistical approaches) in studies of similar nature so that the observations could be compared and broad remedial measures could be evolved (Ganesh *et al*., 2014).

Microbial source tracking (MST) is used to assess recreational water quality and associated human health risk, and total maximum daily load allocations. Human sewage pollution is among the greatest concerns for human health due to: the known risk of exposure to human waste and the public and regulatory to reduce sewage pollution; however, methods to identify animal sources are receiving increasing attention as our understanding of zoonotic disease potential improves. Relationships among human-associated markers, fecal indicator bacteria, pathogens, and human health outcomes are presented along with recommendations (Harwood *et al*., 2013).

Glucose is one of the most important sources of energy for the animals. Glucose has been studied as an indicator of stress caused by physical factors (Manush *et al.,* 2005). Glucose concentration showed sudden elevation in the first day of experiment then dropped to the minimum concentration in the 7th day. Similar results were observed in Triglycerides concentration in the first day of exposure in the highest concentration of *E. coli* (10^9–10^{10} ml/L *E. coli*). The increase in plasma glucose level may be due to glucogenesis to provide energy for the increased metabolic demand imposed by *E. coli* toxins. An increase in glucose was observed by Haney *et al* (1992) in chum salmon (*Onchorhynchusketa*) with erythrocytic necrosis virus, Martins *et al.* (2008) in *Oreochromis niloticus* experimentally infected with *Enterococcus* sp. and Authman *et al.* (2013) in *Clarias gariepinus* collected from El Rahawy drain(polluted with sewage), Egypt. Previous investigations proved that, heavy metals such as cadmium modulate the metabolism of carbohydrates, causing hyperglycemia by stimulating the glycogenolysis in some marine and freshwater fish species (Levesque *et al.,* 2002).

Wedemeyer and Mc Leary (1981) reported that, high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stressors. A variety of stressors stimulate the adrenal tissues resulting in increased level of circulating glucocorticoids (Partap and Bonga, 1990; Hontella 1996 and Glut and Hanke, 1985) and catecholamine's (Witters, 1991).

Hyperglycemia response in this study is an induction of disrupted carbohydrate metabolism possibly due to enhanced breakdown of liver glycogen and mediated perhaps by adrenocortical hormones and reduced insulin secretory activity. Similar hyperglycemic response has been reported in *Cyprinus carpio* exposed to sublethal concentrations of chromium (Parvathi *et al.,* 2011).

Cholesterol level increased in experimental fish than control in all study period except the last day of exposure. The highest concentration was observed in (10^9 – 10^{10} ml/L *E. coli*) in the 5th day of exposure. Cholesterol is an essential structural component of membranes and the precursor of all steroid hormones. The concentration of cholesterol may increase due to the liver failure causing the release of it into the blood. The present results agree with the reports of many investigators (Yang and Chen, 2003 and Parvathi *et al.,* 2011).

Triglyceride level was extremely higher than control in the 1st day of exposure in the 3rd phase. Elevated plasma triglyceride (TG) levels leads to an atherogenic lipoprotein phenotype consisting of high plasma TG levels, low high-density lipoprotein cholesterol (HDL-C) levels and small dense low-density lipoprotein (LDL) particles (Bernis and Krauss, 2002).

The intestine of *O. niloticus* infected with *E. coli* showed degeneration of mucosa and submucosa, necrosis of villi, hyperplasia of goblet cells, infiltration of WBCs, hemorrhage, fragmentation and necrosis of muscular layer beside degeneration of serosa.

Bacteria can enter the fish's body through the gills, skin or gastrointestinal tract (Du Hamel, 2007). *E. coli*, from sewage-fed and conventional ponds were compared, water and fish organs from conventional ponds contained about two orders of magnitude more bacterial cells, reports from Calcutta hospitals that most enteric and other infectious diseases in this region are caused by these bacteria. Significant linear correlations were found between concentrations of these bacteria in pond water and their recovery from several tissues of the fish (Pal and Gupta, 1992).

Bacterial load was higher in the gut contents than in skin, gills and muscle. Detritivorous fish spp. had a higher bacterial count than the filter feeders. Bacterial load was reduced during the depuration period (20 days in fresh water) of the fishes. The fish-sauce preparation examined revealed the complete elimination of MOs (Balasubramanian *et al*., 1992).

Mandal *et al.* (2009) measured the density of *E. coli* in different organs of Nile tilapia. The highest density of *E. coli* was measured in the intestine and gills. El Refaey (2013) isolated bacteria from different fish species (Tilapi, catfish and mullets) collected from Lake Manzalah. Bacterial examination of fresh water fish displayed that tilapia sp. was the highest sp. burden with bacterial isolates. This may be due to that tilapia sp. was genetically suitable to be infected with bacteria. The intestine of catfish showed increased number of goblet cells with severe congestion of submucosal blood vessels and hemorrhage with heavy leucocytic cells infiltration.

Al Yahya *et al.,* (2018) experimentally infected *Oreochromis aureus* with *Aeromonas hydrophilia*, a bacterium that damages intestine, results in hemocyte aggregation. Stratev *et al.,* (2015) noted that the most histopathological damage caused by *Aeromonas hydrophilia*, was seen in the epithelium of liver and kidney followed by the intestines and heart.

Lazar *et al.* (2011) carried out bacteriological and histological examination of different organs of ciprinides infected with *Aeromonas* sp. bacteria. Histological examination of intestine showed lymphatic infiltration, vacuolar dystrophy of epithelial cells of villi and thickening through diffuse hyperplasia. These results nearly agree with our results and with the results of Tornazo *et al.,* 2005 & Robert and Moeller, 2012.

In conclusion, the present investigation revealed that exposure of *O. niloticus* to *E. coli* caused disorder in metabolism and induced pathological changes in intestine and which impede absorption of food stuff finally caused mortality in the highest concentrations. It is recommended to prevent fishing in seas or rivers polluted with sewage or usage of sanitation in farms.

ACKNOWLEDGEMENT

The authors like to extend their appreciation to the owners of Ba Ghanem fish farm, Jeddah KSA for their perfect transport of Nile Tilapia fish samples.

REFERENCES

[1] Al Yahya, S.; Ameen, F.; Al-Niaeem, K.; Al- Sa'adi, B.; Hadi, S. and Mostafa, A. (2018): Histopathological studies of experimental *Aeromonas hydrophilia* infection in blue tilapia, *Oreochromis aureus*. Saudi J. Biol. Sci., 25: 182-185.

[2] Apun, K.; Asiah, M.; Yusof and Jugang K. (1999): Distribution of bacteria in tropical freshwater fish and ponds. Int. J. of Environ. Health Res., 9(4): 285-292.

[3] Authman, M. M. N.; Ibrahim, S. A., El- Kasheif, M. A. and Gaber, H. S. (2013): Heavy metals pollution and their effect on gills and liver of the Nile Catfish *Clarias gariepinus* inhabiting El- Rahawy drain, Egypt. Global Vet., 10(2): 103- 115.

[4] Balasubramanian, S.; Rajan, M.R. and Raj, S.P. (1992): Microbiology of fish grown in a sewage-fed pond. Bio resource Tech., 40 (1):63-66.

. Berneis K.Kand Krauss R.M.(200): Metabolic origins and clinical significance of LDL heterogeneity. J. Lipid Res.;43:1363–1379 [5]

. Bernet, D.; Schmidt, H.; Meier, W.; Burkhardt, P. and Wahli, T. (1999): Histopathology of fish: proposal for a protocol to access aquatic pollution. J. Fish Dis., 22: 25- 34. [6]

[7] Borgattaa, B.; Kmet, N. and Relloc, J. (2012):*E. coli* O104: H4 outbreak and hemolytic- uremic syndrome. Med. Intensiva., 10: 1016.

[8] DuHamel,D.(2007):http://fish.Suite101.com/article.cfm/identifying_fish_diseases#ixzz0YQumYJTC

[9] Dumas, B.T. and Biggs, H.G. (1972): Standard Methods of clinical chemistry. Academic press, New York, USA, pp: 175.

[10] El- Refaey, A. M. E.(2013): Studies on major bacterial diseases affecting fish; Tilapia *Oreochromis niloticus*, Catfish *Clariasgariepinus* and mullets in Port Said, Egypt with special references to its pathological alterations. Researcher, 5(2): 5-14.

[11] Ganesh, T.; Rakhesh, M.; Raman, A.V.; Nanduri, S.; Moore, S. and Rajanna, B.(2014):Macrobenthos response to sewage pollution in a tropical inshore area. Environ. Monitor. & Assess. .186: 3553- 3566.

[12] Glut, G. and Hanke, W. (1985): A comparison of physiological changes in carp, *Cyprinus carpio* induced by several pollutants at sublethal concentrations. The dependency on exposure time. Ecotoxicol. Environ. Saf., 9: 179- 188.

[13] Greenlees, K.; Machado, J.; Bell, T. and Sundlof, S. (1998): Food born microbial pathogens of culture aquatic species. Vet. Chin. North Am. Food Anim. Pract., 14(1): 101- 112.

[14] Haney, D. C.; Hursh, D. A.; Mix, M. C. and Winton, J. R. (1992): Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. J. Aquat. Anim. Health, 4(1): 48- 57.

[15] Harwood, V. J.; Staley, C.; Badgley, B.D.; Borges, K. and Korajkic, A. (2013): Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. Federation of European Microbiological Societies (FEMS) Micro. Rev., pp.1-40.

[16] Hontela, A. (1996): Effect of acute and subacute exposure to cadmium on the inter renal and thyroid function in rainbow trout, *Oncorhynchus mykiss.* Aquat. Toxicol., 35: 171- 182.

[17] Lazar, M.; Vulpe, V.; Guguianu, E.; Lazar, R. Rotaru, A.; Rimbu, C. and Oprean, O. Z. (2011): An episode of bacterial hemorrhagic septicemia with supra-acute course in ciprinides in a farm in Iasi country, Romania. Bull. UASVM Vet. Med., 68(2): 203- 208.

[18] Levesque, H. M.; Moon, T. W.; Campbell, P. G. C. and Hontela, A. (2002): Seasonal variation in carbohydrate and lipid metabolism of yellow perch *(Percaflevescens)* chronically exposed to metals in the field. Aqut. Toxicol., 60(3-4): 257- 267.

[19] Mandal, S.C.; Hasan, M.; Rahman, M. S.;Manik, M. H.; Mahmud, Z.H. and Islam, M.D.S.(2009): Coliform Bacteria in Nile Tilapia, *Oreochromis niloticus* of Shrimp-Gher, Pond and Fish Market. World J. Fish and Mar. Sci., 1(3):160-166.

[20] Manush, S. M.; Pal, A. K.; Das, T. and Mukherjee, S. C. (2005): Dietary high protein and vitamin C mitigate stress due to chelate claw ablation in *Macrobrachium rosenbergii* males. Comp. Biochem. & Physiol. A, 142(1): 10- 11- 18.

[21] Martins, M. L.; Mourino, J. L. P.; Amaral, G. V.;Vieira, F. N.; Dotta, G.; Jatoba, A. M. B.; Pedrotti F. S.; Jeronimo, G. T.; Buglione- Neto, C. C. and Pereira- Jr., G.(2008):Hemotological changes in Nile tilapia experimentally infected with *Enterococcus* sp. Braz. J. Biol., 68(3): 657- 661.

[22] McFarland (2009): Turbidity Standards, Performance Standards for Antimicrobial Susceptibility Testing, Nineteenth Informational Supplement. CLSI, document M100-S19.

[23] McLellan, S.L.; Boehm, A.B. and Shanks, O.C. (2013): Marine and Freshwater Fecal Indicators and Source Identification. Infect. Dis., PP.199-235.

[24] Medema, G. (2013): Microbial Risk Assessment of Pathogens in Water. Environ. Toxicol., pp.361-401.

[25] Pal, D. and Gupta, C. D. (1992): Microbial pollution in water and Its Effect on Fish. J. Aqua. Anim. Heal., 4 (1): 32- 39.

[26] Partap, H. P. and Bonga, S. E. W. (1990): Effect of water- born cadmium on plasma cortisol and glucose in the cichlid fish, *Oreochromis mossambicus.* Comp. Biochem. Physiol., 95: 313- 317.

[27] Parvathi, K.; Sivakumar, P.; Ramesh, M and Sarasu (2011):Sublethal effects of chromium on some biochemical profiles of the fresh water teleost, *Cyprinus carpio*. Inter. J. App. Biol. Pharm. Tec., 2(1): 295- 300.

[28] Plumb, J. A. (1997): Infectious diseases of striped bass. In striped bass and other Morone culture (ed. By Harrel, R. M.), pp. 271- 313.

[29] Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol., 28(1): 56-63.
[30] Robert, B. and Moeller, J. R. (2012): Bacterial diseases of fish, Cichlid- forum.com.
[31] Stratev, D.; Steov. S.; Vashin, I. and Daskalov, H. (2015): Some varieties of pathological changes of pathological changes in experimental per infection of carps *(Cyprinus carpio)* with *Aeromonas hydrophilia*. J. Aquacult. Eng. Fish
[32] Res., 1: 191- 202.
[33] Toranzo, A. E. T.; Margin~os, B. and Romalde, L. (2005): A review of the main bacterial fish diseases in mariculture systems. Aquaculture, 246: 37- 61.
[34] Trinder, P. (1959): Determination of blood glucose using 4-Aminophenazone. J. Clin. Pathol., 22: 246.
[35] Wang, M. and Lu, M. (2016): Tilapia polyculture: a global review. Aquac. Res. 47: 2363- 2374.
[36] Wedemeyer, G. A. and McLeay, D. J. (1981): Methods for determining the tolerance of fishes to environmental stressors. In: Stress and Fish. Edited by Pickering A. D. Academic press, London, 247-275.
[37] Witters, H. E. (1991): Adrenergic response to physiological disturbances in rainbow trout, *Oncorhynchus mykiss* exposed to aluminum at acid. Ph. Can. J. Fish. Aquat. Sci., 48: 414- 420.
[38] Yang, J. and Chen, H. C. (2003): Effects of gallium on common carp, *Cyprinus carpio*; Acute test, serum biochemistry and erythrocyte morphology. Chemosphere, 53: 877- 882.