

## **Impact of water contamination on tilapia (*Oreochromis niloticus*) fish yield**

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**Abstract :** The aim of this work is to study the impact of water contamination on the survival as well as the yield of tilapia (*Oreochromis niloticus*) fish during a complete cycle. The fish culture located in Sharkia Governorate, the source of water was Ismailia channel. Two identical fish ponds were studied. The first pond culture (I) feed with raw water and the other (II) feed with treated water. Water treatment was carried out using a combined sand filter and activated carbon. The treated water was disinfected using 0.4 ppm of chlorine and aeration for 3 to 4 days. The concentrations of bacteriological, physicochemical and heavy metals were determined in all types of samples. The results showed that, total viable bacterial counts at 37°C, 22°C, *E. coli*, *Streptococcus fecalis*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus* were lower in pond (II) compared with pond (I) for both water and fish tissues. In contrast, DO, BOD, TDS and total alkalinity were higher in pond (I) than pond (II), while pH was nearly equal in both of them. Moreover, heavy metals were detected with variable concentrations in pond (I) for water and fish tissues samples. While, Cd and Ni were not detected in pond (II). Also, the growth rate (gm/fish/day) was 2.09 in pond (II) but in other site was 1.27. Similarly, the mortality ratio of fish was 25.4 and 7.24% for I and II ponds, respectively. Consequently, this work provides some advises through water treatment for improving of fish cultures and safety of this type of food to human consumption as well as preservation of the public health.

**Keywords:** Water Pollution of Ismailia canal, Bacteria, water treatment, Physicochemical, Heavy metals, Tissues of fish.

### **Introduction**

Worldwide, the human rely on the fish instead of animal protein intakes (Omojowo and Omojasola<sup>1</sup>). In Egypt, fisheries are very important as a source of animal protein components as well as contribute to agricultural sector product (El-Naggar et al<sup>2</sup>). In order to increase the production of aquaculture, monitoring the quality of both water and fish during a complete cycle is very important. Water quality is an important factor which directly affects the growth rate, fish's health, survival and therefor economic as well as public health.

Some authors (Shelton and Murphy<sup>3</sup>, Arkoosh et al<sup>4</sup> and Joseph and Simeon<sup>5</sup>) demonstrated that, the water pollution (chemically or biological) which used in fish farms increases the likelihood of disease or death among fish, causing a shortage of animal protein in markets as well as economic loss (Olufemi<sup>6</sup>). For instance, the fish infected with *E. coli*, *Vibrio cholera* *Shigella dysenteriae* and, *Mycobacterium tuberculosis* caused dysentery, cholera, shigellosis and tuberculosis, respectively, as well as *Aeromonas*, *salmocida* caused furunculosis of salmonids carp erythrodermatitis for human consumption (Okafor<sup>7</sup> and Austin<sup>8</sup>).

In addition, in Nigeria, Joseph and Simeon<sup>5</sup> recommended that the water which contains bacterial organisms of fecal and industrial pollution must be treated before feeding fish lagoon. Authors reported that, from water contaminated with bacteria (fecal coliforms and fecal streptococci as well as *Salmonella spp.*) might infect fish farm where they detected pathogenic bacteria in mouths, gills and guts of fish. It has to emphasize the treatment of such water to reduce the pathogenic organisms in water and to protect the fish from diseases. Also, other researchers reported that fish or the aquatic environments are sources of bacterial disease which could be transmitted to human beings causing diseases (Hatha Mohamed and Lakshmana-perumalsamy<sup>9</sup> and Novotny et al<sup>10</sup>).

So far there are no microbiological or chemically standards for water used in fish farm ponds and therefore, it is very important to monitor the water quality (De Donno et al<sup>11</sup> and Ali et al<sup>12</sup>). Several studies revealed that, pathogenic microorganism, polluted water and food of fish have adverse effect on fish production (Snieszko<sup>13</sup>, Krkošek<sup>14</sup> and James et al<sup>15</sup>). Austin<sup>16</sup> reported that the presence of organic matter in water increases the pathogenic microorganisms; some of these may cause diseases to fish.

Nora and Diallo<sup>17</sup> reported that in some countries people especially in municipal regions treated raw water (organic contamination) using activated carbon filter to further purify to be reused in fish culture tanks and drinking water. In additions, they reported that bacteria, toxic halo-organic compound (pesticides) and heavy metals which can be removed by using activated carbon and silver nanoparticles.

Ivan<sup>18</sup> in Russia, noticed that, heavy metal were raised in some rivers (Moskva and Oka rivers) and therefore affected on the fish's life causing occurrence of diseases white-eyes bream and liver bream as well as silver bream. This leads to the dead of infected fish. In addition, Marcel, et al<sup>19</sup> in Malaysia, noticed that, from human activity the water in Semantan River and Kenyir Lake were loaded with potential pathogenic bacteria. These bacteria were detected in fish (collected randomly from 30 tilapias) from tissues, brain, eye and kidney, in addition, the authors commented that this type of fish are out break for human consumption.

Some authors (Daskalov<sup>20</sup>, Najiah et al<sup>21</sup>) reported that fatal out comes from fish (tilapia) when infected with *Streptococcus spp.*, *Enterococcus spp.*, *Micrococcus spp.*, *Staphylococcus spp.* *Enterobacteriaceae*, *Aeromonas spp.*, *Pseudomonas spp.* and *Vibrio spp.* Moreover, Heavy metals are effect on the quality of the aquatic environment, with its long-term impact on living organisms epically, some metals which are not biodegradable and accumulated in different organs of human and animals as well as plant (Yang and Chen<sup>22</sup> and Saad et al<sup>23</sup>).

In Egypt, (Lake Qarun), Tayel et al<sup>24</sup> reported that, histopathological organs and muscle composition were found to be damaged due to the contamination of water with heavy metals. So, the researchers recommended that this water must be treated before discharging into the lake because it may affect on fish production and human health.

Moreover, the sources of oxygen gas in water by aeration from rapid movement the surrounding air and as photosynthesis product from watery plants. Some studies (Popma and Masser<sup>25</sup> and Kumar and Pur<sup>26</sup>) reported that tilapia can survive in ponds fish farms at concentration of 0.3 mg/L dissolved oxygen for several hours. Although, the optimum dissolved oxygen concentration ranging from 7 to 8 mg/L. While less or higher than these values possibly causing disease for ponds' fish.

Also, Mohamed et al<sup>27</sup> in Egypt, observed that the higher levels of ammonia on the shore of Nile in Delta area (comes from some factories) causing the deaths of tens of tons of fish. Some researchers reported that, heavy metals (from human activity) and hydrocarbons as well as pesticides materials, are often released into the aquatic environment, these materials due to pollute water and therefore may be an immediate impact for both morbidity and mortality in fish farming (Austin<sup>16</sup>, Moraes and Martins<sup>28</sup> and Ali et al<sup>12</sup>).

Also, in Egypt, Ali et al<sup>12</sup> demonstrated that the present heavy metal in river Nile, (as Cu) with high concentrate for both morbidity and mortality in fish farming, analysis this fish showed its highest value in liver as well as Cu and Cd accumulated on some tissues of fish and therefore harm for humans after consumption. Thus, this study focuses on periodic surveillance of water and fish farm through detection of the pathogens bacteria and some heavy metals as well as physiochemical parameters to protect the public health and national economy as well as ecosystem.

## Materials and Methods

### Area of study

The study was extended for 180 days (during October 2014 until March 2015) in a fish farm on Bellbas desert road. The fish farm away 6.22 km from Cairo Bellbas road, Sharkia governorate, Egypt. Water and fish samples were collected from fish ponds farm (site, N: 30° 18' 16.1"; E: 31° 29' 15.67").

### Aquariums specifications

For the purpose of this study; 2 fish ponds were constructed. The first pond (fish pond I) with dimension of 6 x 15m and depth of 4m. The walls made of stainless with funnel shape base with pore in the bottom. It opened every an hour for 2 to 5 seconds to throw the waste of fish or excess food. Then water were received directly from Ismailia channel through float (pond I), to control the water level inside the aquarium, as well as water in basins were changed completely every 15 days and aeration into pond all time during this work. Another similar pond (fish pond II) but was feed with water treated with sand filter, activated carbon and 0.4 ppm of chlorine, then water stored in tank (diameters, 6 x10 m with depth of 8m, made of fiberglass) for 14 days and then, repeated the same treatment condition before access to pond II.

The feed of fish is a mixture of soybeans, corn, and wheat, every morning (breaking one day every week), the fish were feeding 5gm food / 100gm biomass of fish / day, with decrease gram every 2 months and every basin were contained about from 2500 fish. At starting of this study, the initial weight of a fish was about 8 to 10 grams while the mature weight was ranged from 236.5 to 384.5 grams. The fish were weight every 2 weeks by gram (Lock and Voluntary<sup>29</sup> and FAO<sup>a30,b31,c32</sup>).

### Samples

Every 2 weeks water samples (pond I, and pond II, canal water and storage water tank were collected individually placed in sterilized polyethylene bottles and transported to the laboratory in ice box. Then prepared immediately for bacterial examined, physiochemical and heavy metals analyses (APHA<sup>33</sup>).

Also, every 2 weeks gills and muscles samples [from pond I, pond II and dead or alive fish during this investigation] were examined by parameters previously mentioned.

### Preparing samples for analysis

#### Water

Ten ml of each water sample was subjected to serially dilutions ( $10^{-1}$  to  $10^{-5}$ ) with sterile physiological 0.85% normal saline in deionized water.

#### Fish tissue

Also, the fish body surface was wiped with 70% ethanol, and parts of gills, and muscles were taken from each fish. 10 g of each organ was aseptically transferred in to 90 ml of sterilized 0.85% normal saline, homogenized and centrifuged for 2.5 min at 14000 rpm and then allowed to stand for about five min. Tenfold serial dilution were done (Julshamn<sup>34</sup> and APHA<sup>33</sup>). In addition, all data of bacterial examination from tissues were calculated as cfu/gm.

### Bacteriological analysis

Ten ml of each water sample was subjected to serially dilutions ( $10^{-1}$  to  $10^{-5}$ ) with sterile physiological saline (0.85% wt/vol. NaCl) in deionized water. The poured plate technique and the nutrient agar were used for the enumeration of total bacterial counts at 37°C and 22°C incubation temperatures. MPN technique was used for enumerated total and fecal coliforms (using Luryle Tryptose broth medium and confirmed by used Brilliant Green broth and EC medium) as well as used Eosin Methylene Blue (EMP) agar medium was used for enumerated *Escherichia coli*. Also, MPN technique was used for enumerated fecal streptococci (using Azide Dextrose broth medium and confirmed by Ethyl Violet Azide dextrose broth medium), incubated at 37°C for

24-48 h for both two media (APHA<sup>33</sup>). *Pseudomonas aeruginosa* were estimated (Asparagine broth and confirmed by slant of acitamide agar) by using Most Probable Number (MPN) technique according to APHA<sup>33</sup>.

By using membrane filter technique were counted *Staphylococcus aureus* (Manitol Salt agar), *Salmonella* spp. (Bismuth Sulphate agar) according to APHA<sup>33</sup>.

### Heavy metals analysis

Heavy metals (Cd, Pb, Cu and Ni) in water samples and tilapia fish organs (gills and muscles) were determined using atomic absorption spectrometry (Perkin-Elmer 3110, USA) with graphite atomizer HGA-600, after using the digestion technique by nitric acid according to the standard methods for examination of water and wastewater (APHA<sup>33</sup>). The bio-accumulation factor was estimated according to Authman and Abbas<sup>35</sup> as the following equation:

Bioaccumulation factor = (Pollutant concentration in fish organ (mg/kg)/Pollutant in water (mg/l)).

### Physicochemical analysis of water

Water samples have been subjected to various analyses including pH value and total dissolved solids (TDS) by using portable devices (pH meter model HI 8314 and digital conductivity meter HI2300 Hanna Ins. Romania). Dissolved oxygen (DO) was measured using the modified Winkler method and biological oxygen demand (BOD) with the five-day incubation method. Chemical oxygen demand (COD) was carried out using the potassium permanganate method. Colorimetric methods were used to determine ammonia and nitrite and nitrate (APHA<sup>33</sup>).

### Growth performance parameters

Specific growth rate (SGR) =  $(\ln W_2 - \ln W_1) / t \times 100$

Growth rate =  $(W_2 - W_1) / t$

Where: Ln = the natural log

W<sub>2</sub> = Final weight at certain period (gm.)

W<sub>1</sub> = Initial weight at the same period (gm.)

T = Period (day)

Ratio of relative growth rate (%):  $(W_2 - W_1 / W_1) \times 100$  where W<sub>2</sub> and W<sub>1</sub> is the final and initial weight respectively.

Relative mortality rate (%):  $(M_1 - M_2 / M_1) \times 100$  where M<sub>2</sub> and M<sub>1</sub> is the initial and final counts of fish alive respectively

### Statistical analysis

Data were statistically analyzed using analysis of variance (Freed et al 1936) using the STATISTICA (6.0) computer programs.

### Results and Dissections

Human and natural processes affect water quality, biologically, chemically and physically. Thus, harmfully deteriorates the ecosystems and produce unsuitable water for human usage. The most common contaminants are pathogenic microorganisms, trace metals and toxic chemicals.

The microbiological analysis of canal water, storage water tank, I and II water ponds were analyzed for classical bacterial indicators, and some pathogenic bacteria (Table 1).

**Table (1):-Log average count/100ml of classical bacterial indicators, and some pathogenic bacteria in samples (from canal water, storage water tank, I and II ponds) which collected from fish farm at Sharkia governorate, Egypt, (2014-2015).**

Bacteria	Log averages of cell forming unites / 100ml			
	Untreated water		Treated water	
	Canal water	Pond I	Storage tank	Pond II
TBC <sub>a</sub> (cfu/100ml)	6.4	9.6	3.3	6.2
TBC <sub>b</sub> (cfu/100ml)	6.1	8.9	2.8	4.8
TC(MPN/100ml)	1.7	3.5	ND	2.4
FC(MPN/100ml)	1.1	2.4	ND	1.1
Ec(MPN/100ml)	1.07	2.6	ND	1.4
FS (MPN/100ml)	1.2	2.1	0.2	0.9
SA(MPN/100ml)	0.8	2.4	ND	1.6
Ss(MPN/100ml)	0.4	2.1	ND	0.12
PA(MPN/100ml)	2.2	4.2	0.5	3.4

TBC<sub>a</sub> =Total bacterial count at 37°, TBC<sub>b</sub> =Total bacterial count at 22°C

TC = total coliform, FC = Fecal coliform, ND = Not detected, Ec = *E. coli*,

FS = Fecal Streptococci, SA= *Staphylococcus aureus*, Ss = *Salmonella* spp.

PA =*Pseudomonas aeruginosa*., MPN/100ml= Most probable number-index / 100 ml.

Table (1) shows that the higher log averages count for total viable bacterial count at 37°C and 22°C were recorded 9.6 and 8.9 cfu/100 ml, respectively, in water samples from pond I than other tested sites. While the lower log averages were recorded in water samples from storage water tank in both incubations temperatures. Whereas the higher values of log averages count of MPN-index/100 ml for total coliforms, fecal coliforms, *E. coli* and fecal streptococci count were 3.5, 2.4, 2.6 and 2.1, respectively from pond I water samples. On the other hand, all the previously examined strains (except total bacterial counts) were not detected in water samples from storage tank. Also, *Staphylococcus aureus*, and *Salmonella* spp. were not detected in water samples from storage water tank, while *Pseudomonas aeruginosa* presents in most samples with log averages of 0.5 MPN-index/100 ml. Moreover, *Staphylococcus aureus*, *Salmonella* spp. and *Pseudomonas aeruginosa* were detected in ponds I and II where the log averages were highest in pond I than pond II were recorded 2.4, 2.1 and 4.2 MPN-index/100 ml, respectively. It can be noted that water from canal and pond I were polluted more than other water sites. The source of pollution in pond I may be attributed to the excess of feed as well as waste of fish that increase counts of bacteria in water.

With regard to the results in Table I, *Pseudomonas aeruginosa*, some researchers (Hardalo and Edberg<sup>37</sup>, Devictorica and Galván<sup>38</sup> and Grisey et al<sup>39</sup>) reported that *Pseudomonas aeruginosa* may be able to survive in water for several weeks as well as more resistant to different environmental conditions.

Accordingly, the treatment processes which applied in this investigation play an important role for the improvement of water quality from Ismailia canal. Also, water quality especially total bacterial counts have a stress on fish life. This can be vulnerable to infection by pathogenic bacteria and therefore effect on fish production.

The obtained results were in a good agreement with that of Marcel et al<sup>19</sup> in Malaysia whose studied 3 sites, first from in cage-cultured system in Kenyir Lake, the second from Semantan River and the third from sediment of river Semantan. The predominant bacterial strains isolated from water in site 1 were *Staphylococcus xylosum*, *Staphylococcus lentus*, *Klebsiella terrigena* and *Kocuria varians* (20%), respectively. While, in site 2 were *Staphylococcus lentus*, (30%) followed by *Staphylococcus xylosum*, (20%). Moreover another isolates were detected in water samples like *Klebsiella terrigena*, *Salmonella arizonae* and *Serratia odorifera* with ratio 10% but in site 3 were *Pseudomonas aeruginosa* and *Enterobacter cloacae* with 50%, respectively. On the other hand, 15 tissues of Red hybrid tilapia from each site (1 and 2) were infected. The results of bacterial isolates of site 1 recorded that, *Micrococcus* spp. and *Aeromonas hydrophila* with percentage of 13.64% for both bacteria. While at site 2, *Aeromonas hydrophila* was 23.53%, followed by *Staphylococcus xylosum* and *Staphylococcus caprae* both of them with 11.8%. Siti-Zahrah et al<sup>40</sup> noticed that, diseases from infected fish in tilapia farming in Malaysia can be happened due to water pollution. In addition, the researchers

concluded that, the present pathogenic bacteria in contaminated water have impact on of fish health (Siti-Zahrah et al<sup>40</sup>). Also, they suggested that, the source of infectious diseases in fish may be due to the chicken manure which used as organic fertilizer or goat faces as fish feed in the ponds as well as periodic surveillance of water. Moreover, Mohamed et al<sup>27</sup> in Egypt recommended that, protecting the fish in ecosystems could be improved by the controlling the treatment of wastes from different sources before discharging into River Nile.

In addition, results of this investigation were in a good agreement with Ali et al<sup>12</sup> who studied in Egypt the impact of wastewater on the health of *Oreochromis niloticus* from two sites (80 fish/site) of River Nile (El-Sail Drain) at Aswan Governorate. Bacterial counts were examined in site (I) before drain (N=241 water samples) and site (II) after drain (N=245 water samples). They found that, the averages of total bacterial count at 37°C (cfu/ml), total coliform (MPN/100 ml), fecal coliform (MPN/100 ml), fecal Streptococci (MPN/100 ml), *Salmonella* spp. (cfu ml<sup>-1</sup>) and *E coli* (cfu ml<sup>-1</sup>) were recorded 3.1×10<sup>4</sup>, 21.9×10<sup>4</sup>; 3.5×10<sup>2</sup>, 1.6×10<sup>3</sup>; 0.5×10<sup>2</sup>, 2.75×10<sup>2</sup>; 1.1×10<sup>2</sup>, 1.1 x10<sup>2</sup>; 0.2x10, 0.4x10; and 1.6 x10, 3.5 x10, for two sites, respectively. Authors reported that fish are not fit especially from site II which reservoir untreated wastewater in River Nile at this area and therefore represents serious hazard on consumption.

The results in Table 1 are line with Joseph and Simeon<sup>5</sup> in Nigeria who studied the yield tilapia fish (*Oreochromis niloticus*) on both raw (lagoon) and treated water (pond). The average counts for total bacterial, total coliform, *E. coli*, fecal streptococci, *Staphylococcus aureus* and *Salmonella* spp. were detected in different sites, where in lagoon water samples were recorded as 9.6x10<sup>7</sup>, 9.1x 10<sup>6</sup>, 4.8x10<sup>6</sup>, 2.5x10<sup>6</sup>, 1.4x10<sup>6</sup>, and 2.9x10<sup>6</sup>cfu/100 ml but in water pond water samples were 5.6x10<sup>5</sup>, 0.4x10<sup>5</sup>, 0.3x10<sup>4</sup>, 0.3x10<sup>4</sup>, 0.3x10<sup>4</sup> and 0.5x10<sup>4</sup> cfu/100 ml, respectively. Authors, found that, *Pseudomonas aeruginosa* was detected in all samples tested in both water and fish tissues.

The results (Table 1) demonstrated that it should be a policy taken to reduce the discharge of untreated wastewater into River Nile. This will protect the quality of fish which increases the yield and public health of consumers (Austin<sup>16</sup>, Moraes and Martins<sup>28</sup> and Ali et al<sup>12</sup>).

**Table (2):-Averages log number (cfu/gm) of classical bacterial indicators and some pathogenic bacteria in tilapia fish (alive and dead) organs (gills and muscles) in 2 ponds (I untreated and II treated water) from fish farm at Sharkia governorate, Egypt, (2014-2015).**

Bacteria	Counts of cell unites / (cfu/gm)							
	Gills from pond I		Muscles from pond I		Gills from pond II		Muscles from pond II	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
TBC at 37 <sup>0</sup> C	6.3	9.2	2.1	4.3	3.6	6.6	1.05	2.1
TBC at 22 <sup>0</sup> C	4.1	7.3	1.8	3.5	2.1	4.4	0.8	1.6
T. coliform	1.06	1.4	1.02	1.3	ND	0.4	ND	0.7
F. coliform	1.1	1.3	ND	0.7	ND	0.7	ND	ND
<i>E. coli</i>	6	24	2	9	ND	1	ND	ND
F. Streptococci	0.8	1.01	0.5	0.6	ND	0.2	ND	0.1
SA	1.05	1.08	0.2	0.4	0.3	0.5	ND	0.3
<i>Salmonella</i> spp.	1.07	1.5	ND	1.04	ND	1.08	ND	ND
PA	1.5	1.7	1.09	1.3	0.7	2.04	ND	0.9

**Note:-**TBC=Total bacterial count, T = Total, F = Fecal, ND = Not detected

SA= *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*

The presence of classical bacterial indicators and some pathogenic bacteria in gills and muscles in tilapia fish (alive and dead) in 2 ponds were recorded in Table (2). Data of pond I of alive fish in gills revealed that averages log numbers of cell forming unit (cfu / gm) for total bacterial count at 37<sup>0</sup>C, 22<sup>0</sup>C, total coliform, fecal coliform, *E. coli*, fecal streptococci, *Staphylococcus aureus*, *Salmonella* spp. and *Pseudomonas aeruginosa* were 6.3, 4.1, 1.06, 1.1, 6.0, 0.8, 1.05, 1.07 and 1.5, while gills of dead fish were 9.2, 7.3, 1.4, 1.3, 24, 1.01, 1.08, 1.5 and 1.7, respectively. Moreover, data in pond I of alive fish from muscles for total bacterial count at 37<sup>0</sup>C, 22<sup>0</sup>C, total coliform, fecal coliform, *E. coli*, fecal streptococci, *Staphylococcus aureus*, *Salmonella* spp. and *Pseudomonas aeruginosa* were 2.1, 1.8, 1.02, 0.0, 2.0, 0.5, 0.2, 0.0 and 1.09 while muscles in dead fish were 4.3, 3.5, 1.3, 0.7, 9.0, 0.6, 0.4, 1.04 and 1.3 cfu / gm respectively. On other hand, total

coliform, fecal coliform, *E. coli* and fecal streptococci, as well as *Salmonella* spp. were not found in the gills of alive and dead fish in pond II, while other bacterial strains were present. Vice versa, in muscles of alive fish samples (pond II), only log averages of total bacterial count at 37 and 22°C were present with values 1.05 and 0.8 (cfu/ gm) while under other bacteria examined were not detected. In addition, muscles of dead fish samples (pond II) were not detect fecal coliform, *E. coli* and *Salmonella* spp. but others bacteria examined were detected with values 2.1, 1.6, 0.7, 0.1, 0.3 and 0.9 cfu/gm for total bacterial count at 37°C, at 22°C, total coliform, fecal streptococci, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, respectively.

The high load of bacteria as shown in Table 2 were observed in tissues of fish examined from pond I especially for dead fish samples which detected directly from canal water without any treated. Consequently, fish make as a filter in this pond. On the contrary, the high quality of this investigation were regarded form fish samples which live in pond II which feed with treated channel water 2 times before usage in pond II. Generally, the highest counts of bacteria were observed in gills than muscles at different conditions.

Results in Table 2 were in a good agreement with Ali et al<sup>12</sup> in Egypt who examined tissues of tilapia River Nile fish in 2 sites. They found that in site 1, the averages count of total bacterial count at 37°C (cfu×10<sup>4</sup>/gm), bacteria of Enterobacteriaceae (cfu×10<sup>3</sup>/gm), *E. coli* (cfu/gm) and *Salmonella* spp.(cfu/gm) from mussels and gills were 17, 1; 3, 2; 35, 0 and 1, 19; respectively, while average count for the same parameters in site II were being 80, 240; 4, 19; 419, 3218 and 17, 215; respectively. Authors reported that fish are not fit and impact harmful for human consumption especially site 2 where more pollution from discharges wastewater from human or factories than other site examined. Furthermore, Joseph and Simeon<sup>5</sup> in Nigeria detected total bacterial count, total coliform, *E. coli*, fecal streptococci, *Staphylococcus aureus* and *Salmonella* spp. in different bodies (flesh, gills mouth and gut) of fish in both lagoon and pond where gills samples from lagoon were recorded 1.5×10<sup>7</sup>, 4.3×10<sup>6</sup>, 2.9×10<sup>6</sup>, 3.3×10<sup>6</sup>, 6.0×10<sup>6</sup> and 2.4 ×10<sup>5</sup>cfu/gm, but in pond were recorded 7.1×10<sup>5</sup>, 5.8×10<sup>5</sup>, 0.6×10<sup>5</sup>, 0.3x ×10<sup>5</sup>, 0.2 ×10<sup>5</sup>and 0.3 ×10<sup>5</sup>cfu/gm, respectively. They reported that lagoons higher polluted than ponds where lagoons recipient waste from human activity as well as they demonstrated that fish from these sources are not fit for public health.

Also, this study are line with Dosoky et al<sup>41</sup> in Egypt, when treated water samples (n=27) collected from 5 fish farms by silver nanoparticles (concentrate 0.1 ppm), as antibacterial activity for 5 minutes. They found that, total bacterial counts (10<sup>5</sup> – 10<sup>4</sup>cfu/ml), total coliform (10<sup>4</sup> – 10<sup>3</sup> cfu/ml) and total fecal streptococci (10<sup>2</sup> – 10<sup>3</sup>cfu / ml) were decreased with ratio 85.3, 92.48 and 90.48%, respectively. Moreover, authors concluded that, water used in fish farms must be treated (by silver nanoparticles in form of filter or net system) for count reduction of bacteria and to produce fish free from diseases.

Finally, this work demonstrated that to prevent maturate fish farm from infection with bacterial diseases, it must be treated water which used for aquacultures as well as use some material against bacteria for reduction pathogenic microorganisms which cause great economic and ecological losses (Huicab-Pech et al<sup>42</sup>).

**Table (3):-The ranged values of Physicochemical and trace metals parameters in water samples for 180 days collected from canal water, storage tank, pond I and pond II from fish farm at Sharkia governorate, Egypt, (2014-2015).**

Parameter	Untreated water		Treated water		Permissible level
	Canal water	Pond I	Water tank	Pond II	
pH (range)	7.2 to 7.9	6.9 to 8.5	7.1 to 8.2	6.8 to 8.1	6.5-8.5
TDS (ppm)	934 to 1055	928 to 1114	158 to 182	82 to 121	1000 (ppm)
DO (ppm)	3.2 to 4.4	3.2 to 5.1	6.4 to 7.9	4.5 to 6.1	5-8 (ppm)
BOD (ppm)	3.4 to 4.8	4.5 to 6.2	2.8 to 3.4	3.6 to 4.8	1-5 (ppm)
COD (ppm)	1.06 to 1.08	1.07 to 1.1	0.001 to 0.03	0.04 to 0.09	0.05(ppm)
NO <sub>2</sub> (ppm)	14 to 32	18 to 38	9 to 17	11 to 21	45 (ppm)
NO <sub>3</sub> (ppm))	0.2 to 1.2	0.2 to 1.5	0.1to 0.2	16 to 29	0.2 (ppm)
NH <sub>3</sub> (ppm))	0.052 to 0.086	0.056 to 0.128	0.021 to 0.048	0.051 to 0.058	0.05(ppm)
Cu (ppm)	0.28 to 0.88	0.24 to 0.65	ND	ND	1-2 (ppm)
Cd (ppm)	0.018 to 0.049	0.016 to 0.029	ND	ND	0.003(ppm)
Ni (ppm)	0.0 to 0.001	0.0 to 0.001	ND	ND	0.02 (ppm)
Pb (ppm)	0.019 to 0.043	0.017 to 0.039	ND	ND	0.01 (ppm)

ND = Not detected, BOD = Biological Oxygen Demand, TDS = total dissolve solid,

COD = Carbon Oxygen Demand, DO = Dissolve Oxygen, Cu = copper, Cd = Cadmium, Ni = Nickel, Pb = Lead.

The physicochemical and heavy metal analysis of different water samples (canal water, storage tank, pond I and pond II) is shown in Table 3.

Data recorded in Table 3 showed that, the pH value, TDS, COD and BOD were higher in water samples from pond I which contains canal water than another samples in the experimental site, where the recorded concentration ranged from 6.9 to 8.5, from 928 to 1114, from 4.5 to 6.2 and from 1.07 to 1.1, respectively. On the other hand, the lower DO value ranged from 3.2 to 5.1 was recorded in pond I. Also, the soluble forms of nitrogen; nitrite, nitrate and ammonia showed reduction in storage water tanks samples and the range of concentration was from 9 to 17, from 0.1 to 0.2 and from 0.021 to 0.048 ppm, respectively. While the highest concentration of ammonia ranged from 0.56 to 0.0128 ppm recorded in pond I. This may be due to the bioactivity of microorganisms.

Heavy metals Cu, Cd, Ni and Pb were not detected in water samples from storage tank and pond II and detected in other site of study with range values from 0.28 to 0.88, from 0.018 to 0.049, from 0.0 to 0.001 and from 0.019 to 0.043 in canal water samples while in pond I were from 0.24 to 0.65, from 0.016 to 0.029, from 0.0 to 0.001 and from 0.017 to 0.039, respectively. This work showed that heavy metals and physicochemical analyses were in the permissible level after treatment of canal water for using fish farm according to EOS<sup>43</sup> and Egyptian Standard<sup>44</sup> as well as not affect the fish life through monitoring the water quality in 2 ponds tested.

In addition, the higher concentrations of pH, BOD, and COD as well as ammonia, may correlate to increase in the bacterial count and vice versa (Dosoky et al<sup>41</sup>). On other hand in storage tank and pond II water samples, results may be attributed to treated water pollution (canal water) through sand filter, activated carbon filter and chlorine doses (3-4 ppm).

With regard to data in Table 3, results were not directly correlated with Garish et al<sup>45</sup> this may be increase the pollution now in this mention canal where detected ranged values in raw water samples (n=38) along Ismailia Canal of pH, TDS, Pb, Cd and nitrate where recorded from 7.7 to 8.2, from 227 to 429 (ppm), from 0.65 to 5.88 (ppm), from 0.09 to 0.56 (ppm), and from 1.8 to 5.1 (ppm), but after treated through water treatment plants were from 7.2 to 7.9, from 232 to 412 (ppm), from 1.06 to 13.8 (ppm), from 0.057 to 2.52 (ppm) and from 1.5 to 4.8 (ppm), respectively.

In parallel with this investigation, data in Table 3 are line with Davies and Ansa<sup>46</sup> in Nigeria, who examined some physicochemical parameters water samples in 2 fish farms, one of them (tidal earthen ponds [TEP]), source water is from the New Calabar River which polluted from human activity recorded 6.18, 6.33 ppm, 6.35 ppm and 0.48 ppm while stagnant concrete tanks (SCT), recorded, 6.48, 4.34 ppm, 6.66 ppm and 0.09 ppm, for pH, DO, BOD and ammonia tests, respectively. Authors reported that variation of water quality in these results due to the human activity and natural of this activity effect on life fish in the farms.

On the other hand, results were varied with Ali et al<sup>12</sup> examined 2 sites of water Nile samples in southern of Egypt, they tabled results in site I as, 8.01, 162.2 ppm, 2.04 ppm, 0.83 ppm, 1.2 ppm, 0.012 ppm, 0.128 ppm and 0.091 ppm while site II were recorded 7.82, 165.7 ppm, 1.92 ppm, 1.06 ppm, 2.4 ppm, 0.009 ppm, 0.097 ppm and 0.043 ppm, for pH, TDS, DO, BOD, COD, nitrite, nitrate and ammonia tests, respectively. Moreover, they reported site I less polluted than site II and concluded that the pollution from drains are caused the pollution and negative effect on aquacultures. In addition, according to Egyptian Organization for Standardization (EOS<sup>43</sup>) they observed Cd, Ni, and Pb (except Cu) were higher than the permissible level, and their investigation reported that attributed to the huge quantities of sewage and industrial wastes as well as the leaching of gasoline from the fishery boats and the tour ships. This indicate that the water quality play main role on products marine biologist.

With regard to this work demonstrated that, water treatment which applied in Egypt, it can be reduction some polluted from Nile water like organics, micro-pollutants and heavy metals to protected the ecosystems and public health (Donia<sup>47</sup>).



**Table (4):-Averages values (ppm) of some heavy metals in gills and muscles samples collected from ponds I and II in both alive and dead fish farm.\***

Parameter	Some Heavy metals (ppm) in gills and muscles							
	Gills from pond I		Muscles from pond I		Gills from pond II		Muscles from pond II	
	live	Dead	live	Dead	live	Dead	live	Dead
Cu (ppm)	2.3	5.2	0.8	2.1	ND	0.8	ND	1.0
Cd (ppm)	0.41	0.68	0.40	0.58	ND	0.2	ND	0.1
Ni (ppm)	5.4	8.4	ND	2.1	ND	ND	ND	ND
Pb (ppm)	11.1	18.6	4.2	8.8	2.2	8.4	0.5	1.4
<b>Permissible level (EOS<sup>43</sup>)</b>								
	Cu (ppm)		Cd (ppm)		Ni (ppm)		Pb (ppm)	
	30 mg/kg		2 mg/kg		0.4 mg/kg		2 mg/kg	

\*These averages values are detected from 78 fishes of both dead (n=39) and alive (n=39) from two ponds during 180 days.

Some selected heavy metals (Cu, Cd, Ni and Pb) in fish tissues (gills and muscles) were measured and presented in Table 4 for both alive and dead fishes in ponds I and II. Generally, results showed that, the average values of metals for fish tissues tested in pond I were found to be higher than that recorded in pond II. While heavy metals tested in alive fish samples were lower than in dead fish. Also, the higher average values of heavy metals were detected in gills higher than in muscles. On other hand, Cu, Cd and Ni / ppm were absent while Pb was present in gills and muscles of fish tested from pond II in both alive and dead fishes. Moreover, averages value of Pb was recorded in all fish tissues tested where the lowest value was being 0.5/ppm in muscles of life fish from pond II, while highest value was being 18.6/ppm in gills of fish dead from pond I. Results showed that, the accumulation of different metals depended upon organ of fish and water quality as well as type of metal (Mohamed and Aboul-Ezz<sup>48</sup>). Moreover, increasing the concentration of the heavy metals in the gills and muscles of fish may be kill fishes (Ismail and Saleh<sup>49</sup>).

The results were not agreed with Saeed and Shaker<sup>23</sup> in Egypt, who found that Cu, Cd and Pb in Lake Manzala from samples (n=20) of *Tilapia Spp.* gills were recorded 242.12, 32.22 and 56.12 while, in muscles were 10.36; 48.84 and 10.1 µg/g dry weight, respectively. These results were higher than our investigation; this may be due to the pollution of the aquatic environment by wastes of factories, agricultures or domestic waste which plays a role in health hazards for consumers and fish life. On the other hand, the results were in a good agreement with Omar et al<sup>50</sup> (Egypt, El-Fayoum depression), heavy metals from Fish (gills and muscles) farm of the Faculty of Agriculture, El-Fayoum University (Site 1, sources water from Nile water), and fish from Lake Qaroun (Site 2, El-Wadi drainage canal) which is one of the main drainage canals in El-Fayoum province which used for aquaculture in Qaroun area, as well as fish from farm of Lake Qaroun (site 3, agricultural drainage water as a water source). The mean values (n=8 from every sites) of heavy metals from gills of site 1 were 2.75, 12.95 and 0.53; site 2 were 2.97, 104.29 and 14.25 as well as site 3 were 15.67, 107.57 and 24.55, for Cu, Zn and Pb mg/kg, respectively. Also, they detected Cu, Zn and Pb mg/kg, in muscles in site 1 were recorded 0.66, 8.92 and 0.20 while in site 2 were 13.22, 29.19 and 3.76; site 3 were 9.83, 22.36 and 2.38, respectively. Authors reported that results of heavy metals (Cu, Zn and Pb mg/kg) from samples in all sites showed that, fish examined from site 1 were accepted according to EOS<sup>43</sup> and WHO<sup>51</sup> in food for human consumption but heavy metals in gills and muscles were danger from the other sites tested and may be in the future killing the fishes. Based on their results, authors emphasized that reduction or removal of pollution in Lake Qaroun must be carried out to protect aquacultures life and ecosystem.

The results showed that, water pollution has possible infectious effects on fish and changes on histology of bodies with some diseases causing fish death (Ibrahim<sup>52</sup>).

With regard to the result in Table 4, were found to be lined with Javed and Usmani<sup>53</sup> who observed in India (n=25) a *Mastacembelus armatus* fish collected from rivulet situated (Kasimpur, Aligarh), the accumulation of Cu and Ni (mg/Kg.dry weigh) in gills were being 199.88±0.20 and 200.00±1.73 while, in muscles were recorded 41.36±0.54 and 58.98±0.09, respectively. They concluded that this pollution of heavy metals came from Harduaganj Thermal Power Plant to water, thus the present fish infected with heavy metal therefore affected the fish life.

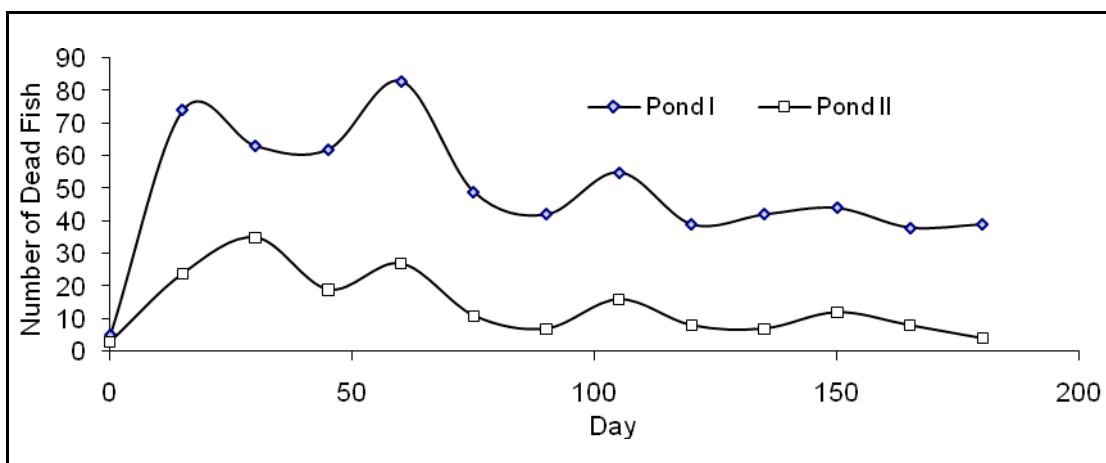
Also, data in Table 4 was agree with Ali et al<sup>12</sup> in Egypt, at Aswan city during autumn 2013 examined 80 fish tissues (gills and muscles) in both 2 sites, site1 before the disposal point of El-Sail drain of gills, Cu, Ni,

Cd, and Pb were 5.2, 8.65, 0.35 and 11.85 ppm respectively, while in muscles were 1.85, 5.2, 1.0 and 7.7 ppm for Cu Ni, Cd and Pb parameters, respectively. While their results for site II (after the disposal point of El-Sail drain) in gills examined of Cu, Ni, Cd, and Pb were 3.05, 7.45, 0.35 and 10.45 ppm, respectively, while in muscles were 3.15, 7.45, 6.4 and 8.0 ppm for Cu, Ni, Cd, and Pb parameters, respectively. Moreover, authors revealed that, the concentration of metal in fish tissues due to sewage and industrial wastes via El-Sail drain. In addition, they reported that, this pollutant affected on fish following on public health for human consumption.

Moreover, results of this investigation were line with that obtained by Noor and Zutshi<sup>54</sup> in South India who studied for one year (2013-2014) bioaccumulation of some trace metals in tissues of fish (gills and muscles) from both Vengaiiah lake (received domestic sewage) and Yellamallappa Chetty lake (received effluents from pharma-industry and other sources) as well as Hebbal fish farm as control. They detected (in gills of fish from Yellamallappa Chetty lake) Al, Cd and Pb were being  $3.81 \pm 0.17$ ;  $0.24 \pm 0.02$  and  $0.26 \pm 0.02$  ppm, respectively, while Al, Cd and Pb in muscles were being  $2.18 \pm 0.14$ ;  $0.41 \pm 0.50$  and  $0.15 \pm 0.04$  ppm. These parameters were not detected in others 2 sites. In addition, authors detected Fe, Cu and Zn in tissues of fish (gills and muscles) from all the tested sites. From phenomenon of these results and sources of pollution as well as exposure period time, authors commented that, trace metals in fish tissues were not high (EOS<sup>43</sup>) but they may break out a potential danger diseases to human consumption and negative impact on public health (Chapman et al<sup>55</sup>).

**Table (5):- The mortality number from fish aquaculture for 180 days, during 2014- 2015 in pond I contains canal water (2500 fish) and treated it in pond II (2500 fish)**

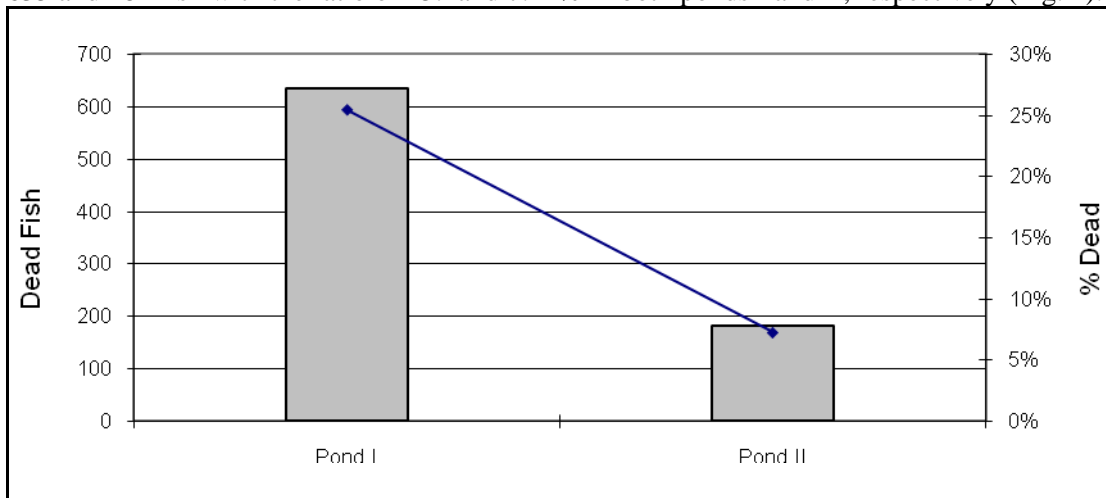
Sample Number	Day No.	Untreated water (Number of dead from 2500 fish) pond I	Treated water (Number of dead from 2500 fish) pond II
1	0	5	3
2	15	74	24
3	30	63	35
4	45	62	19
5	60	83	27
6	75	49	11
7	90	42	7
8	105	55	16
9	120	39	8
10	135	42	7
11	150	44	12
12	165	38	8
13	180	39	4
<b>Total</b>	-	<b>635</b>	<b>181</b>
<b>Death ratio</b>	-	<b>25.4%</b>	<b>7.24%</b>



**Fig. (1):- Mortality of fish in I and II ponds for 180 days from fish farm.**

Tilapia mortality dead of fish from two ponds in this investigation may be from some natural factors like pollution (biologically or chemically) that causes the mortality of fish farms (Porta et al <sup>56</sup>).

Data given in Table (5) and Fig. (1) show the effect of water quality on number of fish culture alive for 180 days during 2014 - 2015 especially in pond II. Data revealed that, the numbers of fish death were reached 635 and 181 fish with the ratio of 25.4 and 7.24% in both ponds I and II, respectively (Fig. 2).



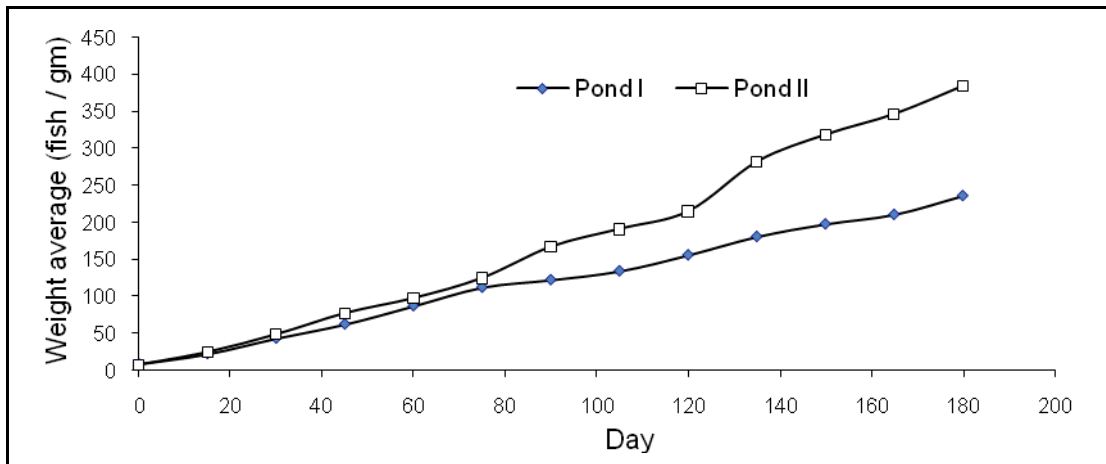
**Fig. 2:- Ratio of mortality of fish in I and II ponds for 180 days from fish farm.**

This data is in agreement with Boyd and Tucker <sup>57</sup> in USA who reported that in fish farm, the mortality were caused due to attacking diseases by microorganisms or toxic condition or excesses of food and wastes from fish. In addition, they found mortality of fish in ponds is about 10 to 20%. In addition, in Tanzania Lamtaneh et al <sup>58</sup> concluded that, to obtain exhalant fish yields product from fish farms, it must monitor and control the quality of water which used in fish ponds for reduction or removal of the factors which affect on the fish farming. Also, results are in line with those obtained by Adebayo and Adesoji <sup>59</sup> who stated in Nigeria from fish pond about higher mortality rate about 40% of survival during 240 days. Moreover, in Bangladesh Islam and Hossain <sup>60</sup> reported that mortality rate decreased 22% when treated surface water used in ponds of fish farms rather than raw water.

In addition, the water quality affected the production traits of fish in aquacultures (Davies and Ansa <sup>46</sup>). Data of weight gain (gm /fish) of fish farms which collected from ponds I and II for 180 days are presented in Table (6) and illustrated by Fig. (3). Results show that, fish weight was increased from 8.7 to 236.5 and 8.2 to 384.5 (gm /fish) for ponds I and II, respectively. Also, the growth rate was recorded 1.27 and 2.09 (gm /fish/day) for ponds I and II, respectively. This means economically, product from pond II (containing treated water) was better than pond I (containing raw canal water).

**Table (6):- Weight average from 10 fishes every 2 weeks for 180 days in I and II ponds from fish farm.**

Sample Number	Day No.	Weight average (fish / gm) from pond I	Weight average (fish / gm) from pond II
1	0	8.7	8.2
2	15	22.4	25.3
3	30	43.5	49.2
4	45	62.5	77.7
5	60	87.3	98.1
6	75	112.4	125.4
7	90	122.5	167.5
8	105	134.5	191.4
9	120	156.2	215.4
10	135	181.15	282.4
11	150	198.2	318.7
12	165	211.1	346.5
13	180	236.5	384.5
<b>Growth rate</b>	-	1.27	2.09



**Fig (3):- Growth rate of Tilapia fish in I and II ponds for 180 days in fish farm.**

These results are in the line with those obtained by Devillera et al<sup>61</sup> in France who found increased growth rate of tilapia fish grow in treated filtered river water (biological filter and 1 mm mesh size) to improve (removal or reduction biological or chemically pollutants) the water quality. Their results of fish weights for one year (gm/fish) were recorded final weight being 366 and 429 (initial weight was 30 gm/fish) from untreated tank and another treated water tank, respectively. The authors noticed that, ratio mortality of fish tested was higher in untreated water tank, than treated water tank where recorded 7.8 and 3.5 (day/fish) respectively, in spite of; growth rate of fish from untreated was higher than another. Also, after filtered river water using a secchi disc in Tanzania Lamtaneh et al<sup>58</sup> observed that final mean weights of growth rate of tilapia fish after 7 months, were from 0.9 to 1.3 and from 0.8 to 1.4 gm/fish/day from four ponds in cities Ruwe and Uba, respectively. In addition, they reported that, the water quality were responsible of fish quality in fish farms. Similarly, in Malaysia Marcel et al<sup>19</sup> studied the role of water contemned on Red hybrid tilapia quality from two sites, first from Kenyir Lake and the second from Semantan River, they found that the weight of fish reached to 250 gm, survived and growth rate in second site were more higher (>250 gm) than fish Kenyir lake. Also, they reported that fish in Semantan River high quality where free from diseases, more resistant for some factors and more survived than fish from Kenyir Lake, this may be to water contamination which come from human activity.

However some studies (Domingo et al<sup>62</sup>, Ferreira et al<sup>63</sup> and Authman et al<sup>64</sup>) reported that, the infected fish with presence of pollutants in fish tissues can affect diseases risk which come from the illegal fish farm on bioactivity on fish and therefor spread heart disease risk. Thus, the use of the contaminated water is expected to be dangerous and adversely effect on the fish and human health especial from illegal fish farming (Alne-na-ei<sup>65</sup>).

## Conclusion and Recommendation

Canal water contains pathogenic bacteria and heavy metals, so by using such water may cause fish diseases and therefore may affect the consumers. On the other hand, all water samples from storage tank were free from bacterial indicators and some examined heavy metals. According to the obtained results from this study pond I was polluted than pond II. The growth rate was higher in pond II than pond I.

This investigation recommended the following:-

- Contemned water may be outbreaks of food poisoning for human through fish which contaminated with bacteria from due to water pollution, or insufficiently heat treated fish .
- It should be avoided environmental factors (physical, chemical and biological) that has harmful effect on fish health causing fish diseases.
- It should be encouraged measures to prevent contamination of water and food to preserve consumer's health.
- To prevent spread of diseases from pathogenic factors (biological or chemical), it should be routinely treatment of water supply and to control of disease vectors and their habitats.

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