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Effect of Microbiological Contamination and Pollution of Water on the Health Status of Fish

Hassan A. Atef [1]; Howayda, M. El Shafei [1]; Mogda, K. Mansour [2]; Samira, A.M. Snosy [3] and Khairy F. Abo-zaid [4].

[1] Dept. of Mycology and Mycotoxins, [2] Biochemistry, [3] Regional laboratory El Dakhla El Wadi El Gadid. and [4] Serology unit, Animal Health Research Institute (AHRI), Dokki, , Agriculture Research Centre (ARC), Giza, Egypt.
atefhassan2000@yahoo.com
howaydaelshafei@yahoo.com

Abstract: Fish has become an increasingly important source of protein and other elements necessary for the maintenance of human healthy. A total of 60 samples includes; (20 samples of each of *Tilapia* fish samples, water samples from water surrounding the collected fish and fish serum), were collected from ponds in El-Wadi -El-Gadid and El-Fayome governorates. Seven genera of molds were recovered from fish samples and four genera from water. Whereas, the *Aspergillus* spp. was recovered from fish and water samples at the rate of (90% and 40%) at the top of all isolated molds. While, the most identified *Aspergillus* spp. from fish and water were *A. flavus* (40% and 10%) and *A. niger* (20% and 15%) and *A. ochraceus* (20% and 5%). While, two genera of yeasts were recovered from fish samples and also from water namely, *Candida* spp. (70 % and 5%) and *Rhodotorula* spp. (70 % and 10%). The incidence rate of aflatoxin in fish was (25%), while, the maximum levels of aflatoxins was (24 ppb) with the mean levels of (13±2.0 ppb). Whereas, no any levels of AFT_s were detected in water samples collected from surrounding water. On the other hand, the bacteria isolated from fish and water samples were identified as *Pseudomonas* spp. (22.5% and 25%), *E. coli* (15% and 35%), *Proteus* spp. (14.2% and 0%), then *Klebsiella* spp. (11.7% and 25%) and *Citrobacter* spp. (10% and 15%), respectively , while *Salmonella* spp. recovered at relatively lower incidence (1.6 and 0%). All species of isolated bacteria were identified biochemically. However, the biochemical examination of water samples indicated that it exhibited extremely high concentrations of iron and ammonia and negatively affected the quality of pond water which caused fish poisoning. The significant increase in AST, ALT, AlP and LDH levels in polluted fish indicates hepatic damage may be due to mycotoxin accumulation which in turn releases these enzymes into the bloodstream. Therefore, all hygienic measures must be performed during catching in clean water, handling, and processing, manufacturing, storage, transportation, marketing of fish to guard the fitness and its hygienic status for consumers.

Key words: Fish, fungi, Enterobacteriaceae, aflatoxins, water pollution, 5% iron toxicity.

1. Introduction

Fish is one of the ideal and most desirable foods which supplies human almost nutritive substances required for his life and it is estimated that more than 30% of fish for human consumption comes from aqua culture **[1].**

Fish come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein **[2].**

The aquaculture in Egypt has become increasingly important in providing an immediate source of animal protein for the progressive country's growing population. Tilapias are widely accepted to Egyptian consumers; represent the species of choice due to its high growth rate, significant tolerance to environmental stresses, ease of reproduction, and its unquestionable market demand. Aquaculture is being

Corresponding Author: Hassan A. Atef

practiced in different forms in Egypt and can tolerate a wide range of environmental conditions **[3].**

Water quality depends on management and on properly designed and constructed ponds that have a dependable water supply. Important water quality variables are dissolved oxygen, pH, total hardness, total alkalinity, iron, hydrogen sulfide content, ammonia, nitrite and salinity. Any disturbed caused by the discharge of untreated sewage, mycotoxin and diverse wastes comprising of heavy metals which are strong biological poisons, due to their persistent nature and cumulative action in their habitats **[4].**

Several studies evaluated the microbial contamination of *Tilapia* fish **[5],** recovered **s**even genera of molds and 2 genera of yeasts from different types of fish. The most commonly isolated mould species in the examined *Tilapia nilotica* were *Alternaria* spp. (90%), followed by *Penicillium* spp., *Cladosporium* spp. and *Candida* spp. (70% for each). Other moulds were recovered in a variable frequency**.** While, water-related diseases of fish continue to be one of the world wide and major health problems **[6].** While**, [7]** reported that both bacteria and fungi are common flora of frozen fish and fish related products during packaging. It is estimated that 80% of all illnesses are linked to use of water of poor microbiological quality. However, the bacterial diseases represent a huge problem among cultured fish in Egypt **[8].**

In addition , the bacterial contamination of fish are a serious threat to aqua culture systems that cause severe damage and mortality rate in Egypt and large economic losses among fish **[9, 10].** The bacterial species of Enterobacteriaceae are a common water borne bacterium which present in the tissues of apparently normal fish **[11],** also, Enterobacteriaceae are considered as an indicator to sewage pollution and has been reported as pathogen in fish **[12].**

On the other hand, molds, yeasts and bacteria were recorded to constitute a public health hazard due to some mycotoxin and bacterial toxin production such as aflatoxin, ochratoxin, patulin and zearalenone toxin and others. These compounds cause some degree of acute toxicity when given in high amounts and are potential carcinogen, where in developing countries; it appears that there is a direct correlation between dietary aflatoxin intake and the incidence of liver cancer **[13, 14].**

These pollutants alter the natural condition of aquatic medium that causes behavioral changes as well as morphological and biochemical imbalance of aquatic organisms **[15]** and may surpass through the food chain **[16].**

The world health organization has recorded that up to 80% of all diseases in the world is caused by polluted water and inadequate sanitation. Also, the same source listed the disease related to unsafe water supplies low hygiene and sanitation controls like food poisoning which caused by *Salmonella* spp. and *E. coli* **[17].**

Good quality water free from fecal pollution and chemicals in harmful amounts, in addition to water must be odorless, colorless and taste less. A useful way of determining water pollution is to test for the presence of fecal organisms in water **[18, 19].**

In Egypt, the water is used in large scale in farm fish and farm animals, coliforms as criteria of water suitability were reported by **[20, 21].** One of the major issues facing Egypt is the accelerated decline of water quality. The future policy aims to prevent resources of pollutants from discharging the Nile water **[22].**

On the other hand, enzymes activity are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants **[23].** Estimation of enzymes likes' aspartate and alanine aminotransferase (AST, ALT) and lactate dehydrogenase (LDH) are considered useful biomarkers to determine pollution level during chronic exposure **[24].** Hence, the evaluation of blood chemistry parameters in animals is a routine and important tool in clinical practices. This simple approach can provide essential information on the physiological status of the animal **[25].** Whereas, the pond aquaculture is the major type of aquaculture in Egypt where only waste lands are allowed to be used for fish mainly because of their high salt and alkali content and poor drainage **[26].**

Therefore, the present study was carried out to study the mycological, mycotoxicological and bacteriological quality of fish (biochemical and serological identification of some highly pathogenic bacteria (*Salmonella* spp. and *E.coli*)) and water and detection of the mycotoxigenicity of the isolated *A. flavus.* In addition, investigation of the most suitable methods for reduction of fungal and bacterial contamination was undertaken and importance of the isolated organism (Public Health importance).

2. Materials and Methods

2.1. Samples: A total of 60 samples includes; (20 samples of each of *Tilapia* fish samples, water samples from water surrounding the collected fish and fish serum), were collected from ponds in El-Wadi -El-Gadid and El-Fayome governorates. The fish samples were collected in sterile polyethylene

bags; water samples were collected in sterile glass bottles and fish serum for chemical examination, were directly identified and transferred to the laboratory under aseptic condition in ice box, without delay.

2.2. Fungal examination of samples:

2.2.1. The fungal culture medium plates were prepared by using different agars as: Malt extract agar (MEA), Sabouraud dextrose Agar (SDA) and Potato dextrose agar (PDA). Sterilization of the media was done by autoclaving at 121°C at 15 psi for 15 min.

2.2.2. Mycotoxins standard solution for TLC:

Aflatoxins standard B_1, B_2, G_1, G_2 were purchased from (Sigma Chemical Company, St. Louis U.S.A).

2.3. Bacteriological examination of samples:

The samples were prepared as recommended by **[27]** and examined according to the standard methods recommended by **[28].**

2.3.1. For Enterobacteriaceae, the method of preparation acc. to **[27]** as follows, 25 gm of each sample were homogenized in 225 ml of BPW under aseptic condition for 2 min by using sterile homogenizer, then incubated at 37°c for 24 hrs. A loopful from this culture broth were separately streaked on MacConkey agar, S.S. agar then incubated at 37°c for 24 hrs. Suspected colonies streaked on nutrient slope agar and incubated at 37°c for further identification in case of skin swabs, must be sterilize the surface skin by alcohol cotten.

2.3.2..For *Pseudomonas* spp. the technique of sample preparation acc. to **[29, 30]** were sample from fish inoculated in peptone water, then incubated at 37°c for 24 hrs, then streaked on to nutrient and MacConkey agar, incubated at 37°c for 24 hrs. The suspected colonies (pale yellow colons) streaked in nutrient agar for further identification.

2.3.3. Identification of isolated bacteria from samples.

2.3.3.1. Confirmation of *S. aureus* coagulase positive isolates by *Staphylococci* Latex agglutination test: *Staphylococci* were tested using dry spot kit **[30].** A fresh culture grown over night 18-36 hrs incubation was used. A positive result showed agglutination of the latex particle within 20 seconds this indicates the presence of *S. aureus* **[31].**

2.3.3.2. Serological identification of *Escherichia coli* species by agglutination test: Serological identification of the isolates was carried out as described by **[32]** using polyvalent and monovalent antisera (DENKA SEIKEN CO., LTD).

2.3.3.3. Serological identification of *Salmonella* spp. isolates:

Fish internal organs samples were routinely grown in selenite F broth, after incubation at 37°C for 18 hr; a loopful was inoculated on MacConkey agar and Salmonella Shigella agar then serological identification was carried out (SIFIN Institute FürImmun-praparate und NährmedienGmb H Berlin Berliner Allee 317/321,13o88 Berlin, Germany) according to **[33].**

2.4. Isolation and identification of fungi from fish:

2.4.1. Fish tissues from different organs of fish samples were sterilized with 1% alcohol for 1 min and then rinsed with sterilized water. Prepared fish and water samples were inoculated on prepared agar plates. Yeasts and moulds were enumerated by the surface plate method using potato dextrose agar (PDA) **[34].** A sample of 25g was homogenized in 225ml of buffered peptone water (BPW) using Warring Laboratory blender. Sample dilutions were spread onto PDA agar supplemented with (0.05 mg/ml media) chloramphenicol antibiotic to reduce bacterial contamination and incubated at 25-30˚C for 4-7 days. Further purification of mould growth was done on malt extract agar (MEA). Slides were prepared from each colony and stained with 0.05% lacto phenol. Identified of all different mould colonies was done to the genera by macroscopic and microscopic examination of the mycelial growth according to the technique recommended by **[35].** The identification of isolated mould and yeast genera and species was carried out according to **[36, 37].**

2.4.2. Detection and measurement of aflatoxins in fish: Aflatoxins were extracted and measured using thin layer chromatography **(TLC)** method according to **[38].** Fifty grams of ground fish meat sample was extracted with 200 ml of methanol-water mixture (55:45) and filtered. Then 50 ml of the extract was placed in a separating funnel using 50 ml of 10% NaCl and 25 ml of hexane for defatting. Aflatoxins were then extracted with duplicate by 25ml of chloroform and the aqueous lower layer and evaporated to dryness in a steam bath. Aflatoxin dry extracts were dissolved in 3ml of dichloromethane and purified in a glass column packing (22 x 300 mm) of 10g silica gel 60M and 0.5g Na_2SO_4 .The packing of the column was done as described in the **[39].** The column was initially conditioned with 30ml of hexane and 30ml of dichloromethane. Aflatoxins were then eluted with 3 portions of 30ml chloroform-acetone mixture (9:1) and the collected eluate evaporated to dryness on a steam bath. Aflatoxins were then recovered with chloroform and spotted onto silica gel TLC plates before development with chloroform - acetone mixture (9:1). Aflatoxin B_1, B_2, G_1 and G_2 reference standards were spotted across the plates. Long wave UV light at 635nm (FUNA UV Light SL-800G) was used to examine the TLC plates so as to establish the presence of aflatoxins.

2.5. Biochemical investigation of samples:

2.5.1. Fish blood samples: were taken from the caudal vein of each fish as described by **[40].** This blood was collected on anticoagulant-free centrifuge tubes. Serum was obtained by centrifugation of blood at 3.000 rpm for 10 min. Serum samples were then stored at −80°C until the analysis.

2.5.2. Water analyses:

All physical and chemical analyses of water samples were measured according to **[41],** nitrite was determined using colorimetric method and nitrate was determined by reduction method as described by **[42].**

2.5.3. Biochemical investigations of fish sera:

Blood Plasma protein content was determined by Biuret method **[43].** Albumin was evaluated according to the method of **[44]** globulin was determined by subtraction of albumin from total protein **[45].** Enzyme activities were measured calorimetrically using kits supplied by Diamond Diagnostics, AST and ALT were determined according to **[46]** and alkaline phosphatase (ALP) according to **[47].** Serum creatinine was measured using the colorimetric method described by **[48]** while uric acid was measured using enzymatic reaction according to **[49].**

2.6. Statistical Analysis: The obtained date were computerized and analyzed for significance, Calculation of standard error and variance according to **[50].**

3. Results and Discussion

Fish meat fulfill all the desired requirement of human consumption and has long been regarded as nutritive and highly desirable food due to its high quality of animal protein content, richness in calcium and phosphorus and its generous supply of ß- complex vitamins. In addition to the low cost of fish in comparison to the cost of meat.

In the present study (Table, 1) the mycological examination of *Tilapia* fish samples and water surrounding the collected fish that collected from ponds in El-Wadi -El-Gadid and El-Fayome Governorates revealed that seven genera of molds were recovered from fish samples. *Aspergillus* spp. isolated at the rate of (90%) at the top of all isolated mould, followed by *Alternaria* spp. (40%), *Cladosporium* spp. and *Penicillium* spp. (20% for each), *Mucor* spp. and *Rhizopus* spp. (10% for each) and *Fusarium* spp.(5%). Incidence of *Aspergillus* spp. was highest in the fish samples while *Fusarium* spp. was the lowest. While, the most identified *Aspergillus* spp. was *A. flavus* (40%), *A. niger* and *A. ochraceous* (20% for each), while *A. terrus* (10%) was recovered at relatively lower rates.

Whereas, the results in (Table, 1) also showed that two genera of yeasts were recovered from fish samples. The most commonly isolated yeast species in the examined *fish* samples were belong to *Candida* spp. and *Rhodotorula* spp. (70 % for each), while two genus of yeasts were recovered from water surrounding the collected fish (*Rhodotorula* (10%) and *Candida* spp. (5%)).

Table (1): Incidence of mould species in examined samples from ponds in El-Wadi -El-Gadid and El-Fayome governorates.

*Identified mould spp.	Fish. (20)		Surrounding water of fish. (20)	
	No. of+v	%	No. of+v	%
***Aspergillus* spp. :**	**18**	**90**	**8**	**40**
-A. flavus.	**8**	**40**	**2**	**10**
-A. niger.	**4**	**20**	**5**	**15**
-A. ochraceus.	**4**	**20**	**1**	**5**
-A.terrus.	**2**	**10**	**0**	**0**
***Penicillium* spp. :**	**4**	**20**	**2**	**10**
-P. citrinium.	**2**	**10**	**1**	**5**
-P. expansum.	**2**	**10**	**1**	**5**
***Alternaria* spp.**	**8**	**40**	**0**	**0**
***Cladosporium* spp.**	**4**	**20**	**2**	**10**
***Fusarium* spp.**	**1**	**5**	**0**	**0**
***Mucor* spp.**	**2**	**10**	**0**	**0**
***Rhizopus* spp.**	**2**	**10**	**2**	**10**
*** Identified yeast spp.**				
***Candida* spp.**	**14**	**70**	**1**	**5**
***Rhodotorula* spp.**	**14**	**70**	**2**	**10**

Currently, four genera of moulds were recovered from water collected from surrounding pond of the collected fish. The most commonly isolated mould species in the examined water were *Aspergillus* spp.(40%), followed by *Cladosporium* spp., *Penicillium spp*. and *Rhizopus* spp. (10% for each).The most common *Aspergillus* spp. isolates from water were *A. niger* (15%), followed by *A. flavus* (10%) and *A. ochrace*us (5%).

Several studies investigated fungal fish diseases as, **[51]** who recovered 2081 fungal isolates from diseased and apparently healthy fish samples. Isolated moulds belonged to the following genera: *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucor*, *Pencillium*, *Rhizopus*, *Scopulariopsis*, *Paecilomyces* and *Curvularia* from *Oreochromis* sp. and *Clarias gariepinus.*

The most predominant mould genera that have been reported in various dried fish products include the *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp, *Mucor* spp, *Fusarium* spp, *Wallemia* spp, and *Cladosporium* spp **[52].** While, **[53]** stated that **t**he particular yeast species of interest in the spoilage of meat and fish products include *Candida, Crytoloccus, Deboromyces, Hansenula, Pichia, Rhodotorula, Saccharomyces, Sporobomyces, Torulopsi* and *Trichosppora.*

The contaminated feeds, water supply and worker hands used in fish breeding play the essential role in the health status of fish **[54].** Where, the contamination was increased in cases of fish caught from polluted areas **[55].** Fish was subjected to many risks of contamination from different sources during fishing marketing till reaching to consumers. The main sources of fish contamination were water, soil, sewage, workers and equipments. Such contamination may render the product unfit for human consumption resulting in significant economic losses or public health hazard to the consumer **[56].**

Similar results were recorded by **[57, 58, 59]** who recovered five genera of moulds and three genera of yeasts from their examined fish samples. The most frequently isolated moulds belonged to genus *Aspergillus* and the obtained *A. flavus* produced different levels of aflatoxins. Also, **[60]** reported that *Aspergillus ochraceus, A. wentii, A. flavus, A. niger, A. parasiticus* and *A. sydawii* were the predominant moulds isolated from dried salted fish. Thirty seven out of 95 isolates of *A. flavus* and all 5 isolates of *A. parasiticus* produced aflatoxins.

However this finding is not accepted by the **[61]** which recommended that fish must be free from obvious mould growth. Similar results were recorded by **[62, 63, 64, 65]** who detected a higher mold contamination in their investigated samples that were collected from ponds and commercial markets, whenever they were exposed to various factors of contamination.

Moulds were recorded to constitute a public health hazard due to mycotoxin production such as aflatoxins, ochratoxins, patulin and zearalenone **[66, 67].**These compounds caused some degree of acute toxicity when given in high amounts and are potential carcinogen **[68].** However, considerable importance has been attached to aflatoxins because of their carcinogenic, mutagenic and teratogenic nature **[69].** Production of aflatoxins is primarily associated with the growth of *Aspergillus flavus* and *Aspergillus parasiticus* **[70, 14].**

In the present work , the tabulated results in (Table,2) illustrated that the incidence rate of aflatoxin in fish samples collected from El-Fayome Governorate pond was (25%) while , the maximum levels of aflatoxins were (24 ppb), while, the minimum levels in same samples were (8 ppb) with the mean levels of (13±2.0 ppb).

Table (2): levels of aflatoxins in examined samples from pond in El-Wadi -El-Gadid and El-Fayome governorates.

Types of examined samples.	Prevalence of aflatoxins.		Levels of aflatoxins (ppb).		
	No. of +ve. samples.	%	Max.	Min.	Mean ± S.E.
Fish (20) from pond in El-Fayome.	5	25	24	8	13±2.0
Surrounding water (20).	N.D.	N.D.	N.D.	N.D.	N.D.

-No. = number. -µg/kg = ppb. -S.E. = standard error. -N.D.= Not Detected.

Although all present water samples were free from AFT_s, AFT_s in fish samples may be due to feedings. Fish samples collected from pond in El-Wadi -El-Gadid Governorate were also free from AFT_s.

It is clear that the present samples of fish showed aflatoxins more than the permissible limits of 15 ppb according to (WHO) and of 20 ppb (FAO & FDA). Also, from the mentioned results, it is evident that the level of aflatoxin B_1 recovered from the examined fish samples exceeded the permissible limits which were recommended by **[71].** As reported by **[61],** fish must be free from obvious mould growth and free of its toxins. However, the detectable aflatoxin residues were recorded in fish samples by different authors such as **[54]** who detected varied amounts of aflatoxins in fish flesh of retail smoked and salted fish.

The aflatoxins have also been found in livers, kidneys and other tissues in animal, birds and fish in feeding trials with aflatoxin contaminated diets. Levels were highest in livers and kidney with only trace amounts occurring in heart, muscle and adipose tissues **[72].**

However, the growth of the *Aspergillus* spp. and the production of aflatoxins are dependent on factors such as the fungal strain, competing flora, substrates, temperature and relative humidity conditions **[73, 13].** The *Aspergillus flavus* is the main source of aflatoxins, the most important mycotoxins in the world's food supplies. Aflatoxins are produced in nature only by *A. flavus, A. parasiticus* and the recently described *A. nomius*. Aflatoxins are both acutely and chronically toxic to animals and human. They produce four distinct effects: acute liver

damage; liver cirrhosis; induction of tumors; and teratogenic effects. Epidemiological data early suggested a link between human liver cancer and aflatoxins **[36].** Aflatoxins are a group of closely related heterocyclic compounds of which six are most common there are four main aflatoxins, B_1, B_2, G_1 and G_2. Of these AFT_s, B_1 and G_1 occur most frequently and in largest amount **[72].**

On the other hand**, [74]** detected a high concentration of aflatoxin B_1 in salted fish which ranged from 450 to 980 µg/ kg. While, **[54]** detected mycotoxins particularly aflatoxin B_1, in higher levels in muscle tissues of salted fish (25±0.1 ppb) than fresh fish (18±0 ppb). While, **[58]** detected the lower levels of aflatoxins in his investigated fish samples (8.93 µg/ kg). Whereas, **[55, 75],** reported that (60%) of *A. flavus* isolated from frozen fish produced aflatoxins at different levels with the mean levels of (6.6±0.5 ppb). However**, [59]** reported that the isolated *Aspergillus* spp. from smoked fish samples produced lower level of aflatoxin B_1 (50% produced up to 10 ppm).

Regarding, the results of bacteriological examination of same fish and water samples.

Table (3): Prevalence of bacterial isolates in examined samples from ponds in El-Wadi -El-Gadid and El-Fayome governorates.

Bacterial isolates	Incidence of bacteria in different organs of fish (20 samples)														Surrounding water (20)	
	Skin swabs (20)		Muscles (20)		Liver (20)		Spleen (20)		Kidney (20)		Intestine (20)		Total (120)		+ve samples	%
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Total bacterial spp													90	75	20	100
***Pseudomonas* spp.**	6	30	2	10	5	25	4	20	3	15	7	35	27	22.5	5	25
E. coli.	3	15	2	10	3	15	3	15	2	10	5	25	18	15	7	35
***Salmonella* spp.**	1	5	-	-	-	-	-		-	-	1	5	2	1.6	-	-
S.typhimurium	1	5	-	-	-	-	-	-	-	-	-	-	1	0.8	-	-
S. enteritides	-	-	-	-	-	-	-	-	-	-	1	5	1	0.8	-	-
***Proteus* spp.**	4	20	-	-	3	15	3	15	2	10	5	25	17	14.2	-	-
***Citrobacter* spp.**	2	10	-	-	3	15	2	10	2	10	3	15	12	10	3	15
***Klebsiella* spp.**	2	10	-	-	3	15	3	15	2	10	4	20	14	11.7	5	25

Percentage of samples acc. to total examined samples.

The current results in Table (3), showed that the bacteria isolated from fish samples were identified as *Pseudomonas* spp. (22.5%), *E. coli* (15%), *Proteus* spp. (14.2%), then *Klebsiella* spp. (11.6%) and *Citrobacter* spp. (10%), respectively , while *Salmonella* spp. Recovered at relatively lower incidence (1.6%). Similar findings to these results were reported by **[76],** who isolated *Pseudomonas* spp. (20%), *E. coli* (18.2%), *Proteus* spp. (10.2%), *Klebsiella* spp. (10.8%) and *Citrobacter* spp. (6.3%) and *Salmonella* spp. (6.2%). On the other hand, different rates of incidence from our findings were (4%), *E. coli* (8%), *Klebsiella* spp. (8%) and *Citrobacter* spp. (12%) and *Salmonella* isolates (16%).

On the other hand, 4 strain of *E. coli* (26.6%), 3 strains of *Pseudomonas* spp. (20%), *Klebsiella* spp. have 3 strains (20%), while *citrobacter* spp. (13.3%), these results were nearly similar results obtained by **[77].** These results also were higher than that observed by **[21, 78],** who recovered *E. coli* (10.2%), *Klebsiella* spp. (2%), *Citrobacter* spp. (11%).

Serious many diseases can be caused by water contaminated with feces or washed into rivers or any water sources as indicators of fecal pollution **[17].** Whereas, well water and surface water are at risk of contamination as indicated by the higher level of most bacteriological parameters detected by coliforms bacterial count **[79, 80].**

In the present study, the data showed in table (3) showed that 80% of water samples were contaminated with bacteria, while, the illustrated results in table 4 revealed the recovering of *E.coli* (35%), *Pseudomonas* spp. and *Klebsiella* spp. (25% for each) and *Citrobacter* spp. (15%), respectively. These results were nearly similar that obtained by **[77].** Also these results are higher than the observed by **[21, 78],** who recovered *E.coli* (10.2*%),* *Citrobacter* spp. (11%) and *Klebsiella* spp. (2%), respectively.

The presence of the enteric pathogen isolated from fish and water are an indicated faecal and environmental pollution, also, due to state of poor hygienic measure and low sanitary condition **[12].**

In addition, fish that catches from ponds for human consumption are contaminated with several types of microorganisms due to many factors related to environment especially contaminated water which influence microbial contamination. They added that, methods of catch, on-board handling fishing, and vessel sanitation processing and storage condition play additional role on the health status of fish **[81].**

Whereas, the isolation of *Pseudomonas* spp. from fish samples in this study, which may be of potential pathogen bacteria as spoilage organism for human food and decrease its quality. A parallel results were previously mentioned by **[82, 83],** who identified *pseudomonas* as good spoilage index.

Currently, the isolation of these enteric organisms indicated faecal and environmental pollution. These supported the finding of **[84],** who isolated pathogenic and potential pathogenic organism from tap water orientated for Nile River, also, these confirms the findings of **[83, 85],** who isolated similar organisms from fish and fish products.

In the present work, all species of isolated bacteria were identified biochemically that confirmed the microscopically and cultural characteristics. The biochemical reaction of isolated enteric pathogen organism, show that, (Indol test) was positive for *E. coli* and *Citrobacter* spp., while, (Methyl red test) was negative in *Klebsiella* spp. and *Pseudomonas* spp. , the (vogus- proskaur) test was positive only with *Klebsiella* spp.. Whereas, the (S. Citrate reaction.and Urea utilization reaction) was positive in *Klebseilla* spp., *Proteus* spp. and *Pseudomonas* spp. also, (T.S.I reaction) are positive (A/Ak with or without H_2S and gas) in *Salmonella* spp. and *Proteus* spp. All isolated are negative of (oxidase test) except *Pseudomonas* spp. and are motile except *Klebsiella* are non motile.

The significance of biochemical reaction is the important methods for isolation and identification of microorganisms as recommended by **[28, 86].**

Additionally, the serological identification of isolated serovars of *Salmonella* from the present fish samples as (2) serovars, which are *S. Typhimurium* and *S. Enteritidis*. Where, *S. Typhimurium* is the most common isolated from fish causing food poisoning and represents about 50-60% of such causes **[87].** Furthermore, **[88]** reported that the cases of food poisoning outbreak due to *S. typhimurium.* The presence of *Salmonellae* as entero-pathogens in fish may affect the hygienic condition which due to un satisfactory methods during catching, handling and marketing of fish **[89].**

Currently, the presence of different strains of *E. coli* gave an indicator about sewage pollution and causes food poisoning and haemorhagic enterocolitis in human due to eating the improperly processed fish meals **[90].**

In general, the Enterobacterieaceae were considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish **[12].** The pathogenic strains of Enterobacteriaceae causes serious disease in fish **[91],** it is a common water borne bacterium which present in tissues of apparently healthy fish **[11]** and may cause serious outbreaks of disease with mortalities of fish when the fish exposed to environmental stress as high temperature, poor water quality **[92]** and **[93].** Some human pathogen such as *Salmonella* spp., *E. coli* and *Kalebsiella* spp. had been found to survive and multiply in the gut and tissues of fish that render the fish acting as potential vector of human diseases **[94].** Also, the isolation of most pathogenic organisms as *Salmonella spp., E. coli* and potential pathogenic organis as *Kalebsiella* spp., *Citrobacter* spp. and *Proteus* spp. which isolated from fish give an indicator about environmental faecal pollution of fish **[95].**

In the present study, the biochemical examination of water sample of outlet pond El-Wadi -El-Gadid revealed increased concentration of total solid, ammonia, and iron compared to typical values found apparently healthy pond in El-Fayome governorate and in **[96, 97, 98, 99, 100]** as table (4). The obtained results indicated that the findings caused fish poisoning in pond due to it exhibited extremely high concentrations of iron and ammonia and negatively affected the quality of pond water.

Table (4): Mean values of chemical analysis of water samples from ponds in El-Wadi -El-Gadid and El-Fayome governorates.

Items	El-Wadi -El-Gadid pond water	El-Fayome pond water	EOS: (2005 a, b, c,d,e)
T.s g/l	2.1	1.65	Up to 1.5
Ph	7.2	7.00	7-7.7
Ca mg%	74	85	100
P mg%	-ve	-ve	0.3
Mg mg%	28	22	15-20
Chlorid mg/l	180	220	Up to 250
Sulphate mg/l	150	209	250
Nitrate mg/l	0.12	0.01	0.02
Nitrite mg/l	60	38	50
Silca mg/l	11	42	50
Ammonia mg/l	0.84	0.21	0.04
Iron mg/l	1.16	0.29	0.50
Chlorine mg/l	0.84	0.65	Up to 1
Free chorine mg/l	0.31	0.33	0.50

EOS= Egyptian Organization for Standardization.

The detected toxicants may accumulate in various tissues which lead to changes in the enzymatic activities. In fish, the liver act as an important organ for uptake, accumulation, biotransformation and excretion of toxicant **[101].** The liver function tests like analysis of serum, AST, ALT, AlP and LDH are widely used to demonstrate liver function or toxicant-induced hepatotoxicity **[102].** In the present study, as in table (5) the significant increase in AST, ALT, AlP and LDH levels in polluted fish indicates hepatic damage may be due to mycotoxin accumulation which in turn releases these enzymes into the bloodstream.

Table (5): Mean values of some biochemical analysis of serum fish samples

Chemical Tests.	El-Fayome pond fish.	El-Wadi -El-Gadid pond fish.
AST µ/l	18.55 ± 0.91	35.22 ± 0.81***
ALT µ/l	14.43 ± 0.51	25.32 ± 0.57***
ALP µ/l	25.43± 0.66	45.06± 0.73**
LDH µ/l	460.23±21.55	582.03±53.42*
Creatinine mg/dl	0.24±0.007	0.26±0.016
Blood urea nitrogen mg/dl	40.13± 0.66	45.17± 0.99**
T.protein g/dl	3.14 ± 0.18	2.57 ± 0.08*
Albumin g/dl	1.56 ±0.03	1.27 ±0.09**
globulin g/dl	1.59± 0.03	1.32± 0.03***

Results are expressed as means ± SEM (n =15), student't' test.

The damage and severity of the organ is dependent on the type of toxicant and duration of exposure **[103]. [104]** linked the increased activity of ALP and LDH in fish to the increased catabolic tissue breakdown in Milano macrophage centers.

[105] showed increased amounts of iron in the gills, kidney, and liver of fish was reflected in increased plasma enzyme activity (ALT, AST).

[106, 107] reponed that exposure of rainbow trout, brown trout and juvenile carp to the liver toxin cyanobacterial caused a dramatic increase in the enzymes ALT and AST released into the bloodstream (an indication of severe liver trauma).

The present elevation of serum blood nitrogen level in all treated groups may be due to the gill dysfunction which leads to disturbance in the diffusion of urea between the blood and the water. An elevated elevation of blood urea nitrogen (BUN) is not indicative of renal disease but it mostly associated with gill and liver diseases **[108].** The present elevation of serum creatinine level may be due to the renal damage.

Proteins of blood serum are a fairly labile biochemical system, precisely reflecting the condition of the organism and changes taking place in it under the influence of internal and external factors.

Regarding the effect of the studied polluted pond on serum total protein, albumin and globulin, the obtained data showed a significant decrease in serum total protein, albumin and globulin compared with their matched healthy groups.

Measurement of total protein, albumin and globulin, in serum or plasma is of considerable diagnostic value in fish, which it relates to general nutritional status as a result of polluted water pond with iron, ammonia and mycotoxins **[109].**

The decrease in serum protein levels may be value for energy production during pollutant toxicity and/or due to other several pathological processes including renal damage and elimination in urine, decrease in liver protein synthesis, alteration in hepatic blood flow and/or plasma dissolution **[110].** Also the iron toxicity includes its role in DNA and membrane damage. Vertebrate have shown that high cellular concentration of iron may cause cell degeneration **[111].**

In addition, mycotoxin has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction have been reported by **[112]** including disruption of normal cell function by inhibiting RNA, DNA, and protein synthesis; inhibition of cell division; stimulation of ribotoxic stress response; and activation of mitogen-activated protein kinases **[113].** Thus, the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis **[114].**

4. Conclusions: Fish are highly perishable and variations in quality due to differences in species, environmental water habitats, feeding habits, in addition, they can also function as carries of several microbial and other health hazards. The greatest risk to human health is due to consumption of raw or insufficiently processed fish or fish products. Fresh water fish are subjected to the risk of contamination with various pathogens from different sources, primary during the presence in aquatic water environment and secondary often harvested through transportation and marketing as well as storage. Such contamination may render these food articles unfit for consumption or even harmful to human consumer. Also, potential public health hazard due to the unhygienic nature of fish handlers to contamination by pathogenic microorganisms. Although, moulds and bacteria may be present without producing any toxin, the presence of the toxigenic fungi and bacteria increases the risk for mycotoxin and bacterial toxin production. In addition, the contamination of fish with some entero-pathogenic bacteria may give an indication about bad sanitary condition exposure of fish during catching till reach to the markets resulting in both Public Health hazard and economic losses. The study suggests that major impact on water quality and fish of the lakes, particularly concerning heavy metals. Heavy metal concentrations in lake and pond water and muscles of Tilapia fish increased compare with the maximum permissible concentrations for human intake.

The results of these study concluded that a greater of contamination of water sources and health education to explain the importance of clean water health sanitation and hygiene.

Periodic sanitary control of raw water should be carried to establish the risk level of epidemic water, the survey should be include the inspection site, evaluation of water supply system and bacteriological analysis of water pollution can offer be identified and measures and taken to prevent future contamination.

There is need for periodic surveillance of water, sediment and tissues of fish to detect the pathogens of paramount importance to aquaculture industry.

Therefore, all hygienic measures must be performed during catching, handling, processing, manufacturing, storage, transportation, marketing of fish and different stages of preparing of human food to minimize the prevalence of the pathogens and to prevent the fungal contamination and to prevent the reach of moulds and their toxins to safe the human health.

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Prof. Dr. Atef Abd El-Aziz Hassan Mohamed, the Former Head of Mycology and Mycotoxins Department, Animal Health Research Institute, Dokki, Cairo, Egypt. He gained B. Vet. Med Sci. (1986); Master of Vet Med. Sci., Microbiology (1990) and PhD of Veterinary Med. Microbiology (mycology, bacteriology and immunology)(1994)(Faculty of Vet. Med. Cairo University, Egypt). The author also gained a Diploma in Clinical biochemistry, Zagazig Univ.(Egypt) (2002). He participated in Supervision on the Master and Ph.D. thesis (Approved) (42 thesis till now) and Published 68 research articles in field of mycosis and mycotoxicosis in animal, poultry and human food of animal origin. Nine Books and 7 Articles in same field were published. From 2007 till 2015, the author awarded by 7 prizes in field of mycosis and mycotoxiocosis in feed, food, animals and birds and their diagnosis and control. The author was nominated for King Faisal international prize in science (biology) 2016. E.mail: atefhassan2000@yahoo.com,
http//www.academia.edu.eg./7688721-AtefHassan
http//www.researchgate.net/puplication/AtefHassan


Dr. Howayda Mohamed El Shafei, B.Vet. Med Sci., Master and Ph.D. of Microbiology, Faculty of Vet. Med., Cairo University, Egypt. Senior researcher Animal Health Research Institute, Department of Mycology &Mycotoxin. E.mail: howaydaelshafei@yahoo.com

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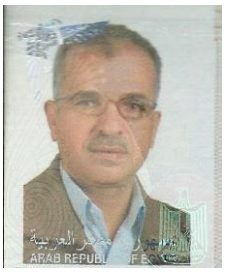
Dr. Khairy F. Abo-zaid, Ph.D. of Microbiology. Faculty of Vet. Med., Cairo University, Egypt. Senior researcher Animal Health Research Institute, Serology unit. E.mail: khairyabozaid@yahoo.com

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Prof. Dr. Mogda, K. Mansour, Ph.D. of Biochemistry. Faculty of Sci., Cairo University, Egypt. Chief researcher Animal Health Research Institute, Department of Biochemistry. E.mail: mogdaabdelsamei@gmail.com

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Dr. Samira, A.M. Snosy, Ph.D. of Biochemistry. Faculty of Vet., Med., Cairo University, Egypt. Senior researcher Animal Health Research Institute, Regional laboratory, El Dakhla- El Wadi- El Gadid. E.mail: samirasnosy@yahoo.com

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