Quality and Safety of Tilapia and Mullet Fish on Shore of Manzala lake and Port Said Markets

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Abstract

Ninety six fish samples of fresh fish, 48 each of Tilapia and Mullet species, 24 samples of each were collected from the on Shore of Manzala Lake and 24 samples of each were collected from Port Said markets, Egypt. The samples were examined for the total aerobic plate count, total coliform count, E.coli count in addition to detection of salmonella and Shigella to evaluate the fish quality and safety.The mean values of TAPC, TC and E.coli in the examined Tilapia fish samples on shore and markets were 3.6 x $10^{2,}$ 7.3x10^1 and 1.5x10^1; 6.8x10^3, 1.4x$10^3$and 2.1x10^1 CFU/g, respectively. While salmonella and Shigella not detected in 25 g in both on Shore and markets fish samples.The mean values of TAPC, TC and *E.coli*in the examined Mullet fish samples on shore and markets were 5.6 x10^2, 4.2x10^1 and 1.2x10^1; 1.4x10^3, 5.3x10^1 and 1.6x10^1 CFU/g, respectively. While Salmonella and Shigella not detected in 25 g in both on Shore and markets fish samples. The significant importance for the bacterial profile revealed that the increased contamination % in TAPC, TC and *E.coli* in Tilapia and Mullet fish samples between on shore and markets were18.9%, 19.2% and 1.4%; 2.5%, 1.3% and 1.3%, respectively.

1. Introduction:

Fish have a highly value of nutrition concerning useful amounts of lipids, protein and essential nutrients. Fish are a high protein source, a low density of calories and high content of omega 3 long chain polyunsaturated F.As (**Tacon and Metian, 2013**).Fish have also a well-balanced amino acid composition; contain high proportions of the vitamins D3 and B12 and the minerals as calcium, phosphorus, iodine, and selenium.Fish also might supply high amount of vitamin A, iron, and zinc if these nutrients are very little in the other sources of them (**Lund, 2013**).

Manzala Lake is considered as one of the most important lakes, which located in the northern part of Egypt and it suffers from more pollutants from different sources such as industrial and agricultural pollutants in addition to the sewage, (**Zaky and Salem 2015**).Manzala Lake also suffers from water pollution and contamination by continues organo-chlorine pollutants(**Abbassy*et al.,* 2003**). The pollution of Manzala Lake has increased the bacterial content particularly that of pathogenic bacteria indicators, such as the fecal coliform, *E.coli* and Enterococci and is shown in the water as in the fish population(**El-Sarangawy 1990**). Manzala Lake is considered one of the largest and shallower lakes that located in the Northern of the Nile delta. The water quality of this lake is brackish which mixed with Mediterranean Sea water through Boughaz El-Gamil opening at Eastern edge of the lake and strait of Sheikh Ali at Western edge East of Damietta Governorates. It link with Suez Canal through a navigation canal (**Abd El-MonsefandKhalifa 2017**). This study was carried out to evaluate the quality of fish and to assess the bacteriological profile of the fish in

Manzala Lake through; sensory evaluation, total aerobic plate count, determination of total coliform count, determination of *E.coli* count, detection of salmonella spp. and detection of shigella spp.

2. Materials and Methods:

Ninety six fish Samples of fresh fish 48 each of Tilapia spp. and Mullet spp. 24 samples of each were collected from the on Shore of Manzala Lake and 24 samples of each were collected from Port Said markets, Egypt. The samples were collected, transferred to laboratory under aseptic condition directly without delay in a clean icebox container.

2.1. Sensory evaluation: The sensory evaluation was carried according to **ES: 7828-1 / 2014** for the following parameters recommended by **ES: 3494(2005**) and the fish grading was carried out according to the Multilingual Guide to EC Freshness Grades for Fishery products (**Howgate*et al.,* 1992**).

2.2.Bacteriological analysis: was carried out according to **ISO 6887-p3/ (2003)**

25 g fish sample was aseptically excised from the dorsal muscle after aseptically removal of the skin. The gills, intestinal area and the anus were covered with sterile cotton wool, drenched in 70% alcohol. The fish meat samples were homogenized thoroughly for insuring sample homogeneity.

Preparation of sample dilutions (ISO 6887-p1:1999)

Enumeration methods:10 g of the prepared fish meat sampleswas added to 90mlof sterile maximum recovery diluents was added. Then the mixture was homogenizedin stomacher 400 lab blenders for 2 minutes to obtain the original homogenate fluid of a dilution rate of (10^{-1}). After that 1 ml was transferred to a series of sterile test tubes containing 9 ml of sterile MRD which was mixed thoroughlyto obtain a 10^{-2} dilution. Further dilutions were obtained up to 10^{-4}.

Detection methods: 25 g of fish meat from the prepared fish samples were weighted under aseptic conditions and added to 225ml of the appropriate primary enrichment according to the method of detection for each microorganism.

Total aerobic plate counts (ISO 4833:2013) One ml from the previously prepared homogenate was transferred on each of duplicate plates, then about 15ml of the standard plate count agar media was poured at 44°c, mixed carefully and allowed to solidify. The inoculated plates were incubated at 30±1°c, for 72± 3 hr. the number of microorganisms / gram of sample are calculated from plates with containing less than 300 colonies; these results were expressed as CFU/g.

Determination of Coliform count (ISO4832:2006) One ml from the previously prepared homogenate were transferred on each of duplicate plates then about 15 ml of VRBL agar medium was poured at 44°cinto each Petri dish , mixed carefully and allowed to solidify. Then 4 ml of VRBL medium was poured onto the surface of the inoculated medium. After that the prepared plates incubated at 37±1°c for 24±2 hr, according to **ISO 7218**.

Determinationof*E.coli* (ISO 16649-2:1999): 25g of fish sample was added to 225ml buffered peptone water, homogenate in stomacher for 3 minute and from the original dilution (10^{-1}) a series of serial dilutions were carried out in peptone saline diluents until 10-4 then 15 ml of Tryptone Bile Glucuronic Agar medium (TBX agar)which previously melted and

cooled at 44°c was added and mix with the inoculums thoroughly. The inoculated plates were incubated at 37 °C for 4 hours and then at 44 °C for 21 hr. The typical blue- green colonies with various shapes were considered ß- glucuronidase +ve*E.coli*.

Detection *of Salmonella* **(ISO 6579:2002):**

Non-selective pre-enrichment: The test sample 25 g was added to 225 ml buffered peptone water and then incubated at 37°c for 18hr.

Selective enrichment:0.1ml of the culture was transferred to a tube containing 10 ml of RVs broth and incubated at 41.5 ± 1°c for 24 ± 3 hr. Another 1ml were transferred to a tube containing 10 ml of MKTTn broth and incubated at 37 ± 1°c for 24± 3hr.

Plating out and identification: Using the cultures obtained previously after incubation from the RVs broth and MKTTn broth the surface of the two dishes containing the first selective plating-out medium XLD agar that was inoculated by means of a sterile loop. All plates were incubated at 37°c for 24hr. and then examined for the blackcentre and a lightly transparent zone of reddish colourcolonies were considered as typical colonies of salmonella.The selective isolated and purified colonies were kept for further biochemical and serological confirmation.

Detection *of Shigella* **spp.(ISO 21567:2004"E"):**25 g of fish sample were added to 225 ml of Shigella broth containing 0.5µg /ml of Novobiocin, the pH were adjusted to 7 ± 0.2 then incubated under anaerobic conditions with caps and closures loose at 41.5 ± 1°c for 16-20 hr.

3. Results and Discussion

3.1.Sensory evaluation:

The data given in table (2) revealed that the non-accepted fish samples according to the sensory evaluation results of Tilapia and Mullet fish species which collected from Port-Said Markets were 4 (16.7%) and 2 (8.3%) grade (A) respectively. Meanwhile 20 (83.3%) and 22 (91.7 %) grade (E) samples of fish species were accepted according to EC **Howgate***et al.,* **(1992)**.

Table (1): Sensory evaluation of Tilapia and Mullet fish samples atPort-Said Markets

* ES: 3494/2005, ES: 7828-1/2014 accepted and non-accepted criteria, Sensory evaluation criteria.

Fish Spp.	Grading **								Accepted Samples*		Non Accepted Samples*	
	E (3)		A (2)		B (1)		C (0)					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Tilapia	20	83.3	4	16.7	0	0	0	0	24	100	0	0
Mullet	22	91.7	2	8.3	0	0	0	0	24	100	0	0

** EC Freshness Grades for Fishery Products **Howgate***et al.,* **(1992)**.

The results revealed that all 48 Tilapia and Mullet fish samples were accepted according to ES: **7828-1 / 2014**. The results were nearly similar to reported with those reported by **Saadia***et al.,* **(2017)**.The non-accepted Tilapia and Mullet fish species collected from Port Said markets samples may attributed to the improper handling after harvesting , unhygienic fish container with un efficient storage condition during transportation and bad storage condition during marketing.

Table (2): Statistical analytical results of bacterial profile of Tilapia fish samples on Shore of Manzala Lake

Bact. Group	Minimum	Maximum	Mean	SE
TAPC	3×10^2	1×10^3	6.3×10^2	$\pm 5.4 \times 10^1$
TC	2.5×10^1	1.5×10^2	7.3×10^1	$\pm 1.1 \times 10^1$
E.coli	1×10^1	4.2×10^1	1.5×10^1	<10

TAPC: Total Aerobic Plate Count. TC: Total coliform.

Bacteriological Indices:

The changes in the bacterial population are considered one of the most important indices which have been used to determine the fish quality and also to evaluate the shelf life of fish.

Bacterial profile of Tilapia fish samples on shore of Manzala Lake:

The result given in table (3), revealed the minimum, maximum, and mean ± SE values of TAPC , TC, *E.coli*, salmonella and Shigella in the examined Tilapia fish samples were 3×10^2, 1×10^3, and $6.3 \times 10^2 \pm 5.4 \times 10^1$; 2.5×10^1, 1.5×10^2 and $7.3 \times 10^1 \pm 1.1 \times 10^1$; 1×10^1, 4.2×10^1 and $1.5 \times 10^1 \pm <10$ CFU/g; 0 and 0, in 25 g respectively. The results for TAPC were less than those reported by **Aman*et al.,* (2017)** and **Al-Harbiand Uddin (2005)** and agreed with the results reported by**Saadia*et al.,* (2017)**. While for TC, These results were agreed with those reported by **Fathy*et al.,* (2015)** and less than reported by **Shankar*et al.,* (2009)**.

Table (3): Statistical analytical results of bacterial profile in examined Tilapia fish samples on shore at Manzala Lake with comparison to Egyptian Standard Specification

Bact. Profile		Not exceed P.L.		Exceed P.L.	
	No.	No.	%	No.	%
TAPC*	24	24	100 %	0	0%
TC*	24	24	100 %	0	0%
E.coli**	24	20	83.3%	4	16.7%
Salmonella*	24	24	100 %	0	0 %
Shigella*	24	24	100 %	0	0%

*According to ES: 3494 (2005)**According to ES: 889-P1 (2009)

The results of *E.coli* were agreed with those reported by **El-Refaey (2013)** and nearly similar to those reported by **Ofred*et al.,* (2016)** and lower than that reported by **Onyango*et al.,* (2009)**. The obtained results for Salmonella were agreed with those reported by **Azza*et al.,* (2012)** and**Chattopadhyay (2000)** while higher incidence of Shigella spp. were reported in examined Tilapia fish species with **Onyango*et al.,* (2009)**.

Table (4): Statistical analytical results of bacterial profile of Tilapia fish samples at Port-Said Markets

Bact. Group	Minimum	Maximum	Mean	SE
TAPC	3.6×10^3	9.6×10^3	6.8×10^3	$\pm 4 \times 10^2$
TC	5×10^2	2.5×10^3	1.4×10^3	$\pm 1.6 \times 10^2$
E.coli	1×10^1	6×10^1	2.1×10^1	<10

The result given in table (4), shown that the bacterial group in all examined on shore fish samples of Tilapia species were not exceed the permissible limit according to **ES: 3494 (2005)**. While 4 (16.7%) of *E.coli* was exceeding the permissible limit (P.L. less than 10CFU/g **ES: 889-P1 (2009)**).

Bacterial profile of Tilapia fish samples at Port-Said markets:

The result given in table (5), revealed the minimum, maximum, and mean ± SE values of TAPC , TC, *E.coli*, Salmonella and Shigella in the examined Tilapia fish samples at Port-Said markets were 3.6×10^3, 9.6×10^3, and $6.3 \times 10^3 \pm 4 \times 10^2$; 5×10^2, 2.5×10^3 and $1.4 \times 10^3 \pm 1.6 \times 10^2$; 1×10^1, 6×10^1 and $2.1 \times 10^1 \pm <10$ CFU/g; 0 and 0, in 25 g respectively. The results of TAPC were less than those reported by **Aman*et al.,* (2017)** and agreedwith those reported by**Saadia*et al.,* (2017)** and **Fathy*et al.,* (2015)** and nearly similar to those reported by **Shankar*et al.,* (2009)**.While the results of TC were agreed with the results reported by **Fathy*et al.,* (2015)** and less than results reported by **Mhango*et al.,* (2010)**.The results of *E.coli* were more than those reported by **Mhango*et al.,* (2010)** and lower than that reported by **Shankar*et al.,* (2009)** and **Onyango*et al.,* (2009)**.Higher incidence of Salmonella spp. in the examined samples of Tilapia was reported by **El-Bayomi*et al.,* (2016)** and **Onyango*et al.,* (2009)**.

Table (5): Statistical analytical results of bacterial profile in examined Tilapia fish samples at Port-Said Markets with comparison to Egyptian Standard Specification

Bact. profile	No.	Not exceed P.L.		Exceed P.L.	
		No.	%	No.	%
TAPC*	24	24	100 %	0	0%
TC*	24	0	0 %	24	100%
E.coli**	24	20	62.5%	9	37.5%
Salmonella*	24	24	100 %	0	0 %
Shigella*	24	24	100 %	0	0%

*According to ES: 3494 (2005). **According to ES: 889-P1 (2009)

The result given in table (6), shown that the bacterial groups in all examined Tilapia fish samples at Port-Said markets were not exceed the permissible limit according to **ES: 3494 (2005)** except in TC result, all examined Tilapia fish samples exceed the permissible limit. While 9 (37.5%) *E.coli* was exceeding the permissible limit (P.L. less than 10CFU/g **ES: 889-P1 (2009)**.

Table (6): Statistical analytical results of bacterial profile of Mullet fish samples on Shore of Manzala Lake

Bact. group	Minimum	Maximum	Mean	SE
TAPC	2.5×10^2	9×10^2	5.6×10^2	$\pm 4 \times 10^1$
TC	1×10^1	1×10^2	1×10^2	<10
E.coli	1×10^1	2.1×10^1	1.3×10^1	<10

Bacterial profile of Mullet fish samples on shore of Manzala Lake:

The result given in table (7) revealed the minimum, maximum, and mean ± SE values of TAPC , TC, *E.coli*, Salmonella and Shigella in the examined Mullet fish samples were 2.5×10^2, 9×10^2, and $5.6 \times 10^2 \pm 4 \times 10^1$; 1×10^1, 1×10^2 and $1 \times 10^2 \pm <10$; 1×10^1, 2.1×10^1 and

$1.3 \times 10^1 \pm <10$ CFU/g; 0 and 0, in 25 g respectively. The results for TAPC were less than those reported by **Aman*et al.,* (2017)** and agreed with those reported by**Saadia*et al.,* (2017)**.

Table (7): Statistical analytical results of bacterial profile in examined Mullet fish samples on shore at Manzala Lake with comparison to Egyptian Standard Specification

Bact. profile	No.	Not exceed P.L.		Exceed P.L.	
		No.	%	No.	
TAPC*	24	24	100 %	0	0%
TC*	24	24	100 %	0	0%
E.coli**	24	16	66.7%	8	33.3%
Salmonella*	24	24	100 %	0	0 %
Shigella*	24	24	100 %	0	0%

*According to ES: 3494 (2005). **According to ES: 889-P1 (2009)

The results of *E.coli* were agreed with those reported by **El-Refaey (2013)**.Higher incidence of Salmonella spp. in the examined samples of Mullet (26.66%) was reported by **El-Bayomi*et al.,* (2016)** and **Onyango*et al.,* (2009)**. Higher incidence of Shigella spp. in the examined Mullet fish species was reported by **Onyango*et al.,* (2009)**.

The result given in table (8), shown that the bacterial groups in all examined on Shore samples of Mullet fish species were not exceed the permissible limit according to **ES: 3494 (2005)**. While 8 (33.3%) *E.coli* was exceeding the permissible limit (P.L. less than 10CFU/g **ES: 889-P1 (2009)**.

Table 8): Statistical analytical results of bacterial profile of Tilapia fish samples at Port-Said Markets

Bact. group	Minimum	Maximum	Mean	SE
TAPC	2.6×10^2	4×10^3	1.4×10^3	$\pm 2.3 \times 10^2$
TC	3×10^1	1.6×10^2	5.3×10^1	<10
E.coli	1×10^1	5.1×10^1	1.6×10^1	<10

Bacterial profile of Mullet fish samples at Port-Said markets:

The result given in table (9) revealed the minimum, maximum, and mean ± SE values of TAPC , TC, E.coli, Salmonella and Shigella in the examined Tilapia fish samples were 2.6×10^2, 4×10^3, and $1.4 \times 10^3 \pm 2.3 \times 10^2$; 3×10^1, 1.6×10^2 and $5.3 \times 10^1 \pm <10$; 1×10^1, 5.1×10^1 and $1.6 \times 10^1 \pm <10$ CFU/g; 0 and 0, in 25 g respectively.

Table (9): Statistical analytical results of bacterial profile in examined Mullet fish samples at Port-Said Markets with comparison to ES (2005)

Bact. profile	No.	Not exceed P.L.		Exceed P.L.	
		No.	%	No.	%
TAPC*	24	24	100 %	0	0%
TC*	24	24	100 %	0	0%
E.coli**	24	16	66.7 %	8	33.3%
Salmonella*	24	24	100 %	0	0 %
Shigella*	24	24	100 %	0	0%

*According to ES: 3494 (2005). **According to ES: 889-P1 (2009)

The obtained results of TAPC were less than those reported by **Aman***et al.,* **(2017)**, agreedwith those reported by**Saadia***et al.,* **(2017)** and nearly similar to those reported by **Calvin** *et al.,* **(2005)**.While the results of *E.coli* were less than those reported by **Onyango***et al.,* **(2009)** and agreed with those reported by **Calvin** *et al.,* **(2005)**.The obtained results of Salmonella were agreed with those reported by **Chattopadhyay (2000)** and **Azza***et al.,* **(2012)**. Lower results of Salmonella spp. in the examined samples of Mullet than those reported by **El-Bayomi***et al.,* **(2016)**.While higher incidence of Shigella spp. in the examined Mullet fish species reported by **Onyango***et al.,* **(2009)**.

The result given in table (10), shown that the bacterial groups in examined Mullet fish samples at Port-Said markets were not exceed the permissible limit according to **ES: 3494 (2005)**. While 8 (33.3％) *E.coli* was exceeding the permissible limit (P.L. less than 10CFU/g **ES: 889-P1 (2009)**.

Table (10): A comparison of the mean values of the examined Tilapia and Mullet fish samples on Shore of Manzala Lake and Port-Said markets

Bact. profile	Tilapia fish			Mullet fish		
	On Shore	Market	Cont.* %	On Shore	Market	Cont*. %
TAPC	$3.6 \times 10^{2\,a}$	6.8×10^{3b}	18.9%	5.6×10^{2a}	1.4×10^{3b}	2.5%
TC	7.3×10^{1a}	1.4×10^{3b}	19.2%	4.2×10^{1a}	5.3×10^{1a}	1.3%
E.coli	1.5×10^{1a}	2.1×10^{1b}	1.4%	1.2×10^{1a}	1.6×10^{1a}	1.3%

*Cont. %: Increased Contamination %

The mean in the same rows contain different symbol were considered significantly difference (p < 0.05). The mean in the same rows contain the same symbol were considered no significantly difference (p > 0.05)

The variations in the above mentioned results for the bacterial groups which obtained either on Shore of Manzala Lake or Port-Said Markets may be attributed to quality of the water in which the fish was harvested, methods of catching, storage condition and duration either on fish vessels, on Shore or Port and Markets and quality and size of ice used in fish storage. Moreover ,the unhygienic wooden and plastic boxes, unhygienic and mishandling by workers' hands, attitude and habits of consumers and marketing and distribution of fish.

A Comparison of the mean values of bacterial profile and the increased contamination % in the examined Tilapia and Mullet fish samples on Shore of Manzala Lake and Port-Said markets

The results given in table (11) revealed the comparison of the mean values of TAPC, TC and E.coli between the on Shore and Market Tilapia fish samples,the increased contamination % between the two locations were 18.9%, 19.2% and 1.4%, respectively. The results given in table (12) revealed the comparison of the mean values of TAPC, TC and E.coli between the on Shore and Market Mullet fish samples,the increased contamination % between the two locations were 2.5%, 1.3% and 1.3%, respectively.

Significantly difference of bacterial contamination between the TAPC, TC and *E.coli* on shore and market fish samples may attributed to contamination of fish from tools and utensils used during catching, during transportation , through improper handling and

distribution of fish in markets.E.coliin fish is considered as a sanitary indicator of potential sewage pollution and indicating pre-harvesting contamination of fish.

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الملخص العربى

جودة و سلامة سمك البلطى و البورى فى بحيرة المنزلة و فى أسواق بورسعيد

ط.ب/ منار عبده عبدالله عبدالله ، د.حسنى عبداللطيف عبدالرحمن و د. تغريد احمد حافظ

قسم الرقابة الصحية على الأغذية ـكلية الطب البيطرى جامعة قناة السويس

تم فحص ٩٦ عينة من كل من أسماك البلطى و البورى بواقع ٤٨ عينة من كل من شاطئ بحيرة المنزلة و أسواق بورسعيد بواقع ٢٤ عينة منهما. وتم فحص العينات للوقوف على قيم متوسطات لكل من العد الكلى للميكروبات الهوائية ، القولونية و الايشريشيا المعويةفى عينات البلطى من شاطئ بحيرة المنزلة و اسواق بورسعيد هي٣.٦ ١٠x٧.٣ ١٠x٢، ١٠x١ و ١٠x١.٥ ؛ ٦.٨ x ١٠ ٣، ١.٤x ١٠ ٣ و ٢.١ x ١٠ ١ خلية / جرام على التوالى. بينما لم يستدل على وجود كل من السامونيلا و الشيجيلا فى ٢٥ جرام فى عينات الأسماك التى تم فحصها من كل من بحيرة المنزلة و الاسواق. وتم فحص العينات للوقوف على قيم متوسطات لكل من العد الكلى للميكروبات الهوائية ، القولونية و الايشريشيا المعوية فى عينات البورى من شاطئ بحيرة المنزلة و اسواق بورسعيد هي٥.٦x ١٠٢، ٤.٢x ١٠ ١ و ١.٢x ١٠ ١ ؛ ١.٤ x ١٠ ٣، ٥.٣x ١٠ و ١.٦x ١٠ ١ خلية / جرام على التوالى. بينما لم يستدل على وجود كل من السامونيلا و الشيجيلا فى ٢٥ جرام فى عينات الأسماك التى تم فحصها من كل من بحيرة المنزلة و الاسواق. و فقم تم مناقشة اهمية المحتوى البكتيري الذي وضح نسبة التلوث الزائدة بين العينات التى تم فحصها من بحيرة المنزلة و أسواق بورسعيد لكل من عينات البلطى والبورى و قد كانت ١٨.٩% ، ١٩.٢% و ١.٤% ؛ ٢.٥%، ١.٣%و ١.٣% على التوالى.

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