

Assessment of microbial and chemical hazards associated with freshwater fishes at different localities in Egypt

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Abstract: Fish make a significant contribution to food and nutrition security in low and middle income countries; however, they are also prone to contamination with a variety of chemical and biological hazards. The aim of this study was to assess the chemical and microbiological quality of Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) from three different locations in Egypt. The results showed that only lead and cadmium were exceeded the national and international permissible limits on the other hand, levels of contamination with microbial pollutants indicated there was a higher levels of coliform, fecal coliform and Staphylococcus counts with a high prevalence for *pseudomonas aeruginosa* in examined fish samples that exceeded the permissible limits and may pose a health hazard on public health. Effect of frying and grilling on reducing the studied heavy metals was also detected with a predominant effect for frying on reducing cadmium, iron, copper and chromium which can improve the quality of fish meat.

[Doha A. Salah Eldin, Shahat A. Ahmed, Laila A. Mohamed, Waleed S. Soliman and Abdelgayed M. Younes. **Assessment of microbial and chemical hazards associated with freshwater fishes at different localities in Egypt.** *Researcher* 2019;11(6):1-12]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). <http://www.sciencepub.net/researcher>. 1. doi:[10.7537/marsrsj110619.01](https://doi.org/10.7537/marsrsj110619.01).

Key words: Microbial hazards, heavy metals, Nile tilapia, Catfish, Egypt, Fry, Grill

1. Introduction

Egypt is a major African country with a population of around hundred million people, depends mainly on fish to fulfill the shortage in red meat and provide the requirements of the animal derived proteins (Morshdy *et al.*, 2013).

Fish is an important aquatic vertebrate whose flesh consumed by human as a valuable fatty acids, vitamins and minerals beneficial to health (Moradi *et al.*, 2011).

Fresh water fish are subjected to the risk of contamination with many pathogens from different sources, primary during their presence in aquatic environment and secondary after being collected through transportation and marketing as well as storage such contamination may make these food articles unfit for human consumption or even harmful to consumers. The dangers accompanied with human pathogenic bacteria in fish can be divided into two groups: bacteria naturally present in the aquatic environment, referred to as indigenous bacteria and those present as a result of contamination with human or animal faeces (Reilley, 1998). These pathogenic bacteria are *Vibrio* spp, *Aeromonas* spp, *Salmonella* spp, pathogenic *E coli*, *Shigella* spp and *Y. enterocolitica*.

In recent years worldwide attention has been paid to the problems of environmental pollution by metals (Malik *et al.*, 2010; Aktar *et al.*, 2011; Qadir and Malik, 2011; Maceda-Veiga *et al.*, 2012). Metals were

of particular concern due to their toxicity and ability to bio-accumulate in aquatic ecosystems (Mohammadi *et al.*, 2011), as well as persistency in the natural environment. Among the metals some are potentially toxic (As, Cd, Pb, Hg, etc.), others are almost essential (Ni, V, Co) and many are essential (Cu, Zn, Fe, Mn) (Biswas *et al.*, 2011). These essential metals can also produce toxic effects when the metal intake is excessively elevated (Tüzen, 2003; Tekin- Özan, 2008).

Heavy metals often enter the environment through human activities, persist in food webs and accumulate in living organisms. In addition to atmospheric sources (Batty *et al.*, 1996), other possible metal sources (Cu, Cd, Pb and Zn) are close to rice fields. Non-essential heavy metals are usually powerful toxins and their bioaccumulation in tissues leads to intoxication, decreased fertility, cellular and of a variety of organs (Oliveira Ribeiro *et al.*, 2000, 2002; Damek-Proprawa and Sawicka-Kapusta, 2003). Essential metals such as copper (Cu), magnesium (Mg), manganese (Mn) and zinc (Zn) have normal physiological regulatory functions (Hogstr and Haux, 2001), but may also bioaccumulate and reach toxic levels (Rietzler *et al.*, 2001). Effects on the environment of these trace metals, mainly from human discharges are not well identified and more information is necessary to better understand the importance of these pollutants, especially in protected areas.

The study aimed to investigate the different pollutants levels from bacteria and heavy metals in Nile tilapia and catfish from three locations in Egypt (El-Hawamdeyya, El-Mariouteya and Kafr-Elsheikh) expressing their hazards on public health and how to control heavy metals present in fish meat by two cooking methods frying and grilling.

2. Material and Methods

Collection of fish samples

A total of random samples of 60 Nile tilapia (*Oreochromis niloticus*) and 60 Cat fish (*Clarias gariepinus*) each fish species was subdivided into 3 groups (20 fish samples in each group) then each group was subdivided to 4 subgroups with 5 composite fish samples in each subgroup collected from three locations in River Nile (El-Hawamdeyya, El-Mariouteya and Kafr-Elsheikh). Samples were transferred to the laboratory in ice box under a complete aseptic condition without undue delay and the soft parts of fish samples be removed and a muscle tissue sample was taken from the dorsal muscle and examined bacteriological and for heavy metal analyses, the positive samples for heavy metals were subjected to further cooking by fry and grill and then analysed for heavy metals again.

Bacteriological examination

1) Bacterial counts

Samples were prepared according to the technique recommended by ICMSF (1978) as follows: ten grams of fish sample were aseptically taken into a sterile homogenizer flask at which 90 ml of sterile (0.1%) Peptone water diluents were added, and homogenized for 2 minutes to provide a homogenate of 10^{-1} dilution. The homogenate was thoroughly mixed, one ml of homogenate was transferred into a sterile test tube containing 9ml of 0.1% Peptone water, and then ten-fold serial dilutions were prepared for Aerobic Plate Count on Standard Plate Count agar, Enterobacteriaceae count on Violet Red Bile Glucose agar, Coliform count using (MPN) 3 dilution technique by LTB Lauryl Tryptose broth (*LTB, Oxoid, and CM451*) and positive results were confirmed using Brilliant Green Bile Lactose Broth (BGLB, Oxoid, CM31), Fecal coliform count confirmed by using Eiosn Methylene Blue Agar (EMBA) (*Oxoid Ltd., Hampshire, England*), Pseudomonas count on Cetermide Fucidin Cephaloridine agar (CFC, Oxoid code CM 559, supplemented with supplement SR 103, Oxoid, Basingstoke, UK) according to (Mead and Adams, 1977) and staphylococcus count by using Mannitol Salt Agar plates according to APHA (1966).

2) Isolation and Identification of the following microorganisms

E.coli, *Pseudomonas auregnusa*, *Staphylococcus aureus* and *Aeromonas hydrophilla* from different fish

samples and identified by using Analytical Profile Index (API) specified for each strain.

Detection of heavy metals in the examined fish samples (Baharom, 2015)

Preparation of fish samples

Five gram of boneless fish muscle tissue was removed using stainless steel knife and was digested to a strong acid digestion ($H_2O_2 + HNO_3$ conc.) mixture at 1: 3 ratios at $150^\circ C$ for 20 minutes and allowed to cool at room temperature. Samples were processed in duplicate and then diluted to a total 50ml with ultra-pure water and filtered through $0.45 \mu m$ micropore membrane filter paper for analyses.

Reagents

All reagents were of analytical reagent grade. Ultra-pure water was used for all dilutions. The element standard solutions from Perkin Elmer that were used for the calibrations were prepared by diluting stock solutions of mg/mL.

Heavy metal analysis

All samples were digested in concentrated HNO_3 and H_2O_2 in a beaker. The samples were then diluted until 50 ml with ultra-pure water. After filtration, the prepared samples were determined for lead, cadmium, iron, copper, zinc, chromium and manganese by using atomic absorption spectrophotometer (AAS) and Inductively Coupled Plasma (ICP-MS). Element standard solution from Perkin Elmer was prepared by diluting stock solutions of 100mg/mL of each element. The concentrations of heavy metals were presented in mg/kg, wet weight (ww) for fish sample.

For cooking fish samples which having heavy metals residues were taken for deboning and mincing with blender then the fish mince was done with blender then the fish mince was used for different cooking methods. For deep frying samples were deep fried in vegetable oil at $180^\circ C$ for 15 minutes until golden brown colour appears. For grilling samples are heat $65^\circ C$ for 10 minutes using electric grill.

Statistical analysis

The results were statistically analyzed by SPSS/20 (SPSS INC., USA), using ANOVA, the presence or absence of significant difference was specified at 95% confidence interval and significance level of $P < 0.05$.

3. Results and Discussion

Microbial Hazards

The presence of foodborne pathogens in fish is related to environmental circumstances and microbiological quality of the water at the fishing site, because the contamination of the water and fish from animal, human and agricultural sources may occur (Feldhusen, 2000; Davies *et al.*, 2001; Hosseini *et al.*, 2004).

Detection of microbial quality in fish is necessary to be aware of and prevent problems related to health and safety. Consequently the present study investigated the presence and the levels of microbiological indicators of the edible part of Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) as well as identification of some foodborne pathogenic bacteria expressing their risk on public health.

As shown in table (1) and illustrated by figure (1) the highest mean values of APC, Enterobacteriaceae count, Coliform and Fecal coliform counts in Nile tilapia was recorded from El-Marioteya with $4.9 \times 10^5 \pm 1.2 \times 10^5$, $2.9 \times 10^4 \pm 2.1 \times 10^4$, $1.5 \times 10^2 \pm 44$ and $1 \times 10^2 \pm 47$ CFU/g respectively. While the lowest mean values were recorded from El-Hawamdeyya with $7 \times 10^3 \pm 2.3 \times 10^3$, 10^2 , 3.15 ± 0.3 and 3 ± 0.17 CFU/g respectively. For *Pseudomonas* and *Staphylococcus* counts of Nile tilapia from Kafr-el-sheikh recorded the highest mean values with $5.6 \times 10^2 \pm 0.5 \times 10^2$ and $1.4 \times 10^3 \pm 9 \times 10^2$ CFU/g and the lowest was detected from El-Hawamdeyya also with mean $1.3 \times 10^2 \pm 0.3 \times 10^2$ and $1.5 \times 10^2 \pm 0.3 \times 10^2$ CFU/g.

Table (2) and Figure (2) illustrated the highest mean values of APC, Enterobacteriaceae count, Coliform and Fecal coliform counts of catfish were recorded from El-Marioteya with $7 \times 10^5 \pm 6.6 \times 10^5$, $5 \times 10^4 \pm 4.6 \times 10^2$, $7.2 \times 10^2 \pm 2.9 \times 10^2$ and $5.9 \times 10^2 \pm 2.9 \times 10^2$ CFU/g respectively, while the lowest mean values were recorded from El-Hawamdeyya with $1.2 \times 10^5 \pm 6 \times 10^3$, $1.9 \times 10^2 \pm 0.5 \times 10^2$, 18.5 ± 0.31 and 3.5 ± 2.5 CFU/g respectively. Catfish samples from Kafr-Elsheikh recorded the highest mean values of *Pseudomonas* and *Staphylococcus* counts with $6.3 \times 10^3 \pm 1.2 \times 10^3$ and $1.5 \times 10^3 \pm 8.5 \times 10^2$ CFU/g but the lowest mean values recorded from El-Hawamdeyya with $2 \times 10^2 \pm 0.5 \times 10^2$ and $6 \times 10^2 \pm 4 \times 10^2$ CFU/g.

According to (ICMSF, 1986) for APC that it does not exceed 10^7 CFU/g so that these results were clearly within the range of good quality fish expect catfish from El-Marioteya that exceeded the permissible limit also the Egyptian Standard Specifications (2005) stated the acceptable limit of Coliform Count that not exceed 10^2 organisms /g, so that catfish samples and Nile tilapia from EL-Marioteya and Kafr-Elsheikh in addition to catfish samples from Kafr-Elsheikh exceeded this limit which indicated the flesh of these fishes have the ability to cause human diseases when consumed or handled, also stated the acceptable limit of Fecal coliform count that not exceed 10 organism /g so that fish samples from El-Hawamdeyya were not exceeded this limit this indicates human health risks due to consumption of Nile tilapia and catfish samples collected from El-Marioteya and Kafr-Elsheikh and for *Staphylococcus*

count not exceeds 10^3 CFU/g so Nile tilapia and catfish samples from Kafr-Elsheikh only exceeds this limit which may constitute a potential health hazards for fish consumers as food poisoning outbreaks.

By comparing our results with the previous studies Ahmed (2007) and Mohamed and Hamid (2011) obtained total viable counts of bacteria from fresh Catfish were 3.7×10^5 and 1.7×10^5 CFU/g respectively, also Jha *et al.* (2010) detected the total bacterial count from *Oreochromis mossambicus* by a mean 1.2×10^4 CFU/g from Siliguri city (India) had nearly similar results. Viji *et al.* (2015) estimated the initial count of Enterobacteriaceae in whole ungutted Catfish was 1.9×10^2 CFU/g which was near to our results also, Yagoub *et al.* (2009) and Begum *et al.* (2010) found the range of Coliform count from Nile tilapia were in range 1×10^2 to 4×10^4 MPN/g and 2.4×10^2 MPN/g respectively. Begum *et al.* (2010) and Budiati *et al.* (2015) evaluated the microbiological quality of catfish and tilapia species and found the ranges of fecal coliform count were 21 MPN/g, 3-43 MPN/g and 3-65 MPN/g respectively which were higher than our results. Begum *et al.* (2010) and Jha *et al.* (2010) obtained *Pseudomonas* count from tilapia species with means 4×10^4 CFU/g and 5.7×10^2 CFU/g which were slightly close to our results. El-Fadaly *et al.* (2016) and Aman *et al.* (2017) found *Staphylococcus* count in Nile tilapia from Egypt with means 10^2 CFU/g and 1.41×10^3 CFU/g which were in agree to our results.

From tables (3) and (4) and Figures (3) and (4) indicated the incidence of the major food poisoning bacteria isolated from Nile tilapia and catfish as for Nile tilapia *E.coli* was highly isolated from 75% of El-Marioteya samples while the lowest was from El-Hawamdeyya with 10%, for *Pseudomonas aeruginosa* was highly isolated from all samples of Kafr-Elsheikh and about 5% of El-Hawamdeyya fish samples. *Aeromonas hydrophilla* was highly detected from 35% of Kafr-Elsheikh samples but it was not detected in El-Hawamdeyya samples, for *Staphylococcus aureus* was highly detected in 60% of Kafr-Elsheikh samples while the lowest was from El-Hawamdeyya with 15%.

For catfish *E.coli* was highly isolated from 50% of El-Marioteya samples while the lowest was from El-Hawamdeyya with 25%, for *Pseudomonas aeruginosa* was highly isolated from all samples of Kafr-Elsheikh and about 75% of El-Hawamdeyya fish samples. *Aeromonas hydrophilla* was highly detected from 20% of Kafr-Elsheikh samples but it was detected in 5% of El-Hawamdeyya samples, for *Staphylococcus aureus* was highly detected in 75% of Kafr-Elsheikh samples while the lowest was from El-Hawamdeyya with 35%.

Heavy metals

Fish being at the top level of the food chain that accumulates large quantities of heavy metals so that this study was investigated on seven heavy metals (lead, cadmium, iron, copper, zinc, chromium and manganese) in the muscle of two commercially fish species Nile tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*) from three locations (El-Hawamdeyya, El-Marioteya and Kafr-Elsheikh) that showed different concentrations, this can be attributed to many factors such as metal concentration, age, size, physiological status, habitat preferences, feeding behavior of the different fish species and this was agreed with (Kalay *et al.*, 1999; Canli and Atli, 2003).

The results recorded in table (5) and illustrated by figure (5) were as follow: for Nile tilapia fish samples the highest mean values of lead, cadmium, copper and chromium concentrations were from El-Marioteya with 0.3 ± 0.1 mg/Kg, 0.5 ± 0.1 mg/kg, 0.9 ± 0.07 mg/Kg and 1.9 ± 0.33 mg/Kg respectively, while the lowest was recorded from Nile tilapia samples from El-Hawamdeyya for copper was 0.04 ± 0.02 mg/Kg and Kafr-Elsheikh for Chromium with mean 0.75 ± 0.1 mg/Kg. On the other hand lead and cadmium were not detected in Nile tilapia fish samples from El-Hawamdeyya. For iron the highest mean value was from Kafr-Elsheikh with 5.6 ± 1.15 mg/Kg and the lowest was from El-Hawamdeyya with 3 ± 0.85 mg/Kg. zinc was detected only in El-Hawamdeyya fish samples with mean 0.017 ± 0.002 mg/Kg and manganese was only present in fish samples from Kafr-Elsheikh with mean 0.04 ± 0.03 mg/Kg.

For catfish samples the highest mean values of lead, cadmium, copper and chromium concentrations were from El-Marioteya with 0.5 ± 0.1 mg/Kg, 1.25 ± 0.4 mg/Kg, 1 ± 0.3 mg/Kg and 1.9 ± 0.1 mg/Kg respectively, while the lowest was recorded from catfish samples obtained from El-Hawamdeyya with mean 0.22 ± 0.15 mg/kg, 0.012 ± 0.002 mg/Kg, 0.04 ± 0.02 mg/Kg but for chromium the lowest was from Kafr-Elsheikh with 0.95 ± 0.13 mg/Kg and zinc was detected from El-Hawamdeyya only with mean 0.023 ± 0.04 mg/Kg and iron was the highest from Kafr-Elsheikh with 7.3 ± 0.4 mg/Kg while, manganese was not detected from any location.

According to the EOSQC (1993) which established a maximum permissible level for lead in fish that not exceed 0.1 mg/Kg so our results were higher than this permissible level. The maximum lead content was found in catfish from El-Marioteya this attributed to catfish (*clarias gariepinus*) are a bottom feeders they are in a close proximity to contaminated sediments that was in agree with Biego *et al.* (2010). For cadmium WHO, 1993 established a maximum permissible limit as 0.05 mg/Kg, so all the fish

samples from El-Marioteya and Kafr-Elsheikh were higher than this level but catfish samples from El-Hawamdeyya was lower than this permissible limit.

For iron FAO (1992) set a permissible for fish that not exceed 100mg/Kg therefore there is no risk of health defects to the popular consumers of the fishes from the studied locations. With respect to copper FAO/WHO (2001) established a limit for it in fish as 30 mg/Kg so that the concentrations of copper in these samples were below this value and regular consumption of fish with such low amounts of copper could not lead to any serious health risk. Concentrations of zinc in all fish samples were below the FAO maximum guidelines of 30mg/Kg for human consumption (FAO, 1983) so the amounts of zinc in the tissues cannot threaten fish themselves as well as human who consume them.

Chromium was below the maximum guideline stipulated by the United States of Food and Drug Administration of 12-13mg/Kg for human consumption (USFDA, 1993), the achieved results also declared that manganese was only found in Nile tilapia fish samples from Kafr-Elsheikh with mean 0.04 ± 0.03 mg/Kg that was below the maximum permissible limit stipulated by (WHO, 1992) of 1mg/Kg.

By comparing our results and those obtained in other studies Khallaf *et al.* (1998), Authman *et al.* (2013), and Bayomy *et al.* (2015) recorded lead from Nile tilapia and catfish from different Egyptian locations which were 0.03 mg/Kg, 0.05 mg/Kg, 0.02-0.03mg/Kg and 0.013-0.02mg/Kg respectively, which were lower than our findings. While, Abdel-Satar and Shehata (2000) obtained lead from *Oreochromis niloticus* from River Nile (Cairo) ranged from 1.5-3.7mg/Kg that were higher than obtained from our results. Mohamed (1993) and Ahmed *et al.* (2010) recorded cadmium in tilapia species with ranges 0.293-0.389mg/Kg and 0.96 ± 0.15 mg/Kg respectively, which were slightly near to our results on the other hand Abdo (2002) recorded cadmium in *Oreochromis niloticus* from Rosetta branch ranged from 1.5-4 mg/Kg which was higher if compared with our results.

In concern to Iron, the results compared with Tiimub and Dzifa (2013) where iron was the dominant heavy metal in the studied fish samples from both Nile tilapia and catfish caught from Densu River (Ghana) with mean 53mg/Kg for Tilapia and 44 mg/Kg for catfish that were higher than our results. Other study by El-Bassir *et al.* (2017) who determined iron in Nile tilapia from Alduba Seen bridge (Khartoum) with mean 0.17mg/Kg and in *Clarias lazera* 0.22mg/Kg which were lower from our results while, Akoto *et al.* (2008) recorded iron in Chin tilapia from Fosu lagoon (Ghana) with a mean 9.98 mg/Kg that was nearly close to our results.

Copper was recorded by Abdulali *et al.* (2003), Kah *et al.* (2015), Hamed *et al.* (2015) and Bahroom *et al.* (2015) from the muscle of Nile tilapia with mean values 1.46-1.69mg/Kg, 0.16-0.27mg/Kg, 0.045mg/Kg and 0.03±0.01mg/kg respectively that were nearly similar to our results. The mean zinc concentration from fish in this study was lower than other studies including Abdulali *et al.* (2003) in Langat River (20.58mg/Kg) and Muiruri *et al.* (2013) in Kenya (28-49.5mg/Kg), while Bahroom *et al.* (2015) and Mohamed and Osman (2014) found low amounts of zinc in *Oreochromis niloticus* 0.43mg/Kg and 0.7mg/Kg respectively. The previous studies reported minimum concentrations of chromium as Ramelow *et al.* (1989) found it in fresh water fish species ranging from 0.15-5.5mg/Kg, Squadrone *et al.* (2013) in the muscle of European catfish with mean 0.06mg/Kg and Mohamed and Osman (2014) in *Oreochromis niloticus* (White Nile) with mean 0.016mg/Kg.

For manganese the results in previous studies were somewhat near to our obtained data at the same fish species for example Bahroom *et al.* (2015) did not record manganese from *Oreochromis niloticus* while Ekeanyanwu *et al.* (2010) found manganese in Nile tilapia with mean 1.97mg/Kg and in Catfish was 1.21mg/Kg.

Effect of cooking on heavy metals concentrations in Nile tilapia and Catfish

The data on the studied heavy metals concentrations in uncooked and cooked fish samples prepared with traditional methods of cooking (frying and grilling) presented in table (6) and (7). From the results of measurements follows that in most analyzed samples of different species the lead concentration remains significantly unchanged as lead possesses a peculiar stability obvious in its very high melting and boiling point which may render its movement away from fish flesh to surrounding cooking medium difficult (Gailtopoulou *et al.*, 2013). The same results were obtained by Ersoy (2011) that there was no significant difference in lead concentrations between raw, fried and grilled African catfish. Contradictory results was obtained by Perello *et al.* (2008) who indicated that the concentrations of lead in Sardine and Tuna were higher in fried samples as compared to the raw samples.

For cadmium in Nile tilapia there is a significant difference ($P<0.05$) between raw, fried and grilled samples and frying caused reduction higher than grilling this observation may be attributed to the ability of fish flesh that act as a permeable membrane most especially under the influence of heat, this permeability may result in appreciable movement of fish water and oil with heavy metals away from flesh

layer into the surrounding cooking medium these in agree with Abowei *et al.* (2011).

In Catfish there is a significant difference ($P<0.05$) between raw, grilled and fried samples but fry and grill nearly have the same effect in aquatic species as previously mentioned by (Gheisari and Rahimi 2016) who stated the presence of many factors that can affect the final concentration of heavy metals after cooking as the initial concentration of heavy metals in the meat, the aquatic species and the type of cooking process.

There is a significant difference ($P<0.05$) for iron among Nile tilapia and catfish between different cooking methods and frying has a predominant effect on its reduction than grilling this attributed to the uptake of frying oil results in complex physicochemical changes of fish composition and texture eventually affecting the distribution of iron between oil and flesh while, from the previous studies Gall *et al.* (1983) didn't observe any significant change in iron content after cooking of fish fillet. Another study Bassey *et al.* (2014) found different effects of processing methods on iron among different fish species as in fried *P.quadrifilis* fish had the highest concentration of iron and the lowest was from the boiled one while, in *C.Ngrodigitatus* grilling and frying gave 34% and 37% decrease in iron concentrations respectively.

For copper there is a significant difference ($P<0.05$) among Nile tilapia and catfish samples but in tilapia the effect of frying and grilling are nearly the same while, in catfish frying has a dominant effect than grilling this was contradictory with Gokoglu *et al.* (2004) who found elevated levels of copper after frying of Rainbow trout while, Gall *et al.* (1983) observed no significant changes in copper content after cooking of fish fillet.

With respect to zinc and manganese there are no significant changes in their concentrations after frying and grilling among the studied fish species this may be attributed to the minimal amounts of the initial concentrations, there are contradictory results obtained for zinc Gokoglu *et al.* (2004) found a reducing effect for frying on zinc content among Rainbow trout.

The decrease in chromium concentration was significant ($P<0.05$) for frying than grilling when compared with the raw one among the studied fish species while, Ersoy (2011) showed higher values of chromium levels after frying African catfish meat. There is a difference among the different cooking methods in decreasing the concentrations of heavy metals depends on the fish species, the initial concentration, time, temperature, cooking medium and the water and protein loss during cooking hence, heavy metals are usually bind with proteins can

increase or deplete elements contents in the fish (Atta *et al.*,1997).

Frying showed a more pronounced effect on heavy metals contents especially for cadmium, iron,

copper and chromium than grilling which may lead to a moderate decrease during process this in agree with Diaconescu *et al.* (2012).

Table (1) Mean values of bacterial counts from Nile tilapia in the three locations (CFU/g)

Bacterial counts	El-Hawamdeyya	El-Marioteya	Kafr-Elsheikh
APC	$7 \times 10^3 \pm 2.3 \times 10^3$	$4.9 \times 10^5 \pm 1.2 \times 10^5$	$1.1 \times 10^5 \pm 9.2 \times 10^4$
Enterobacteriaceae	10^2	$2.9 \times 10^4 \pm 2.1 \times 10^4$	$1.1 \times 10^2 \pm 0.7 \times 10^2$
Coliform count	3.15 ± 0.3	$1.5 \times 10^2 \pm 44$	94 ± 50
Fecal coliform count	3 ± 0.17	$1 \times 10^2 \pm 47$	60 ± 27
Pseudomonas count	$1.3 \times 10^2 \pm 0.3 \times 10^2$	$3.7 \times 10^2 \pm 1.3 \times 10^2$	$5.6 \times 10^2 \pm 0.5 \times 10^2$
Staphylococcus count	$1.5 \times 10^2 \pm 0.3 \times 10^2$	$6.5 \times 10^2 \pm 3 \times 10^2$	$1.4 \times 10^3 \pm 9 \times 10^2$

Table (2) mean values of bacterial counts from catfish in the three locations (CFU/g)

Bacterial counts	El-Hawamdeyya	El-Marioteya	Kafr-Elsheikh
APC	$1.2 \times 10^5 \pm 6 \times 10^3$	$7 \times 10^3 \pm 6.6 \times 10^3$	$2.3 \times 10^5 \pm 1.6 \times 10^5$
Enterobacteriaceae	$1.9 \times 10^2 \pm 0.5 \times 10^2$	$5 \times 10^4 \pm 4.6 \times 10^2$	$1.3 \times 10^3 \pm 3.8 \times 10^2$
Coliform count	18.5 ± 0.31	$7.2 \times 10^2 \pm 2.9 \times 10^2$	$1.9 \times 10^2 \pm 20$
Fecal coliform count	3.5 ± 2.5	$5.9 \times 10^2 \pm 2.9 \times 10^2$	$1.3 \times 10^2 \pm 53$
Pseudomonas count	$2 \times 10^2 \pm 0.5 \times 10^2$	$2.9 \times 10^2 \pm 1.1 \times 10^2$	$6.3 \times 10^3 \pm 1.2 \times 10^3$
Staphylococcus count	$6 \times 10^2 \pm 4 \times 10^2$	$6.5 \times 10^2 \pm 2.4 \times 10^2$	$1.5 \times 10^3 \pm 8.5 \times 10^2$

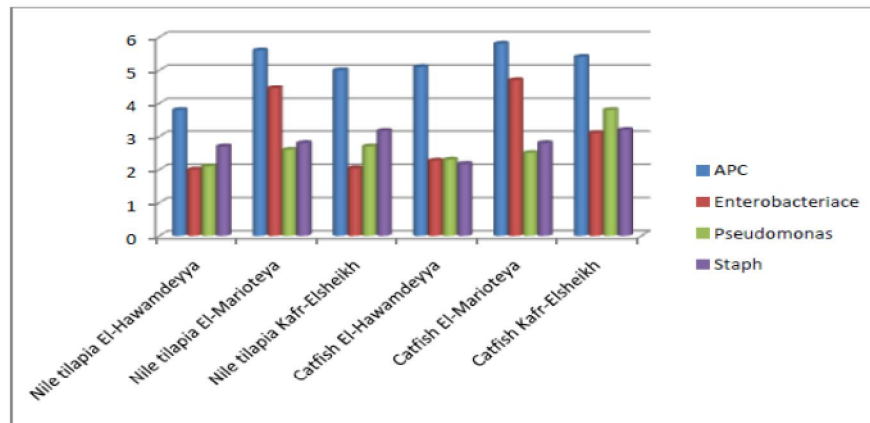


Fig (1) Mean values of bacterial counts from Nile tilapia and catfish in the different locations (log/g)

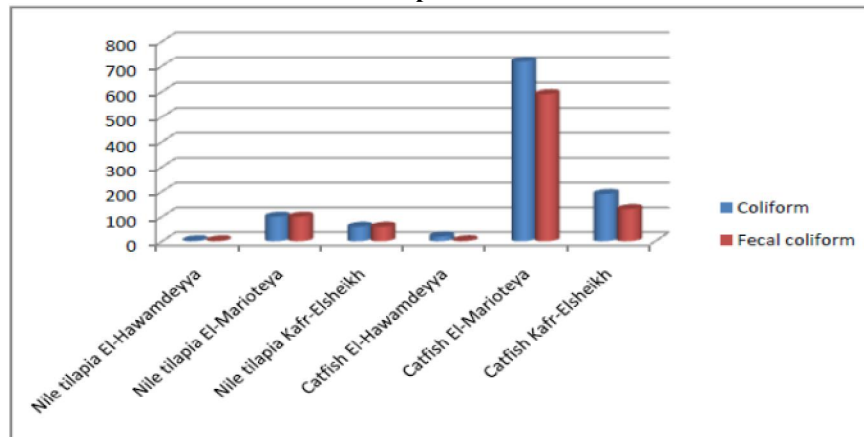


Fig (2) Mean values of Coliform and Fecal coliform counts from Nile tilapia and catfish in the three different locations (log/g)

Table (3) Incidence of the major food poisoning bacteria in examined Nile tilapia samples

Locations	El-Hawamdeyya		El-Mariouteya		Kafr-Elsheikh	
	No	%	No	%	No	%
<i>E.coli</i>	2	10	15	75	5	25
<i>Pseudomonas aeruginosa</i>	5	25	6	30	20	100
<i>Aeromonas hydrophilla</i>	0	0	2	10	7	35
<i>Staphylococcus auerus</i>	3	15	8	40	12	60

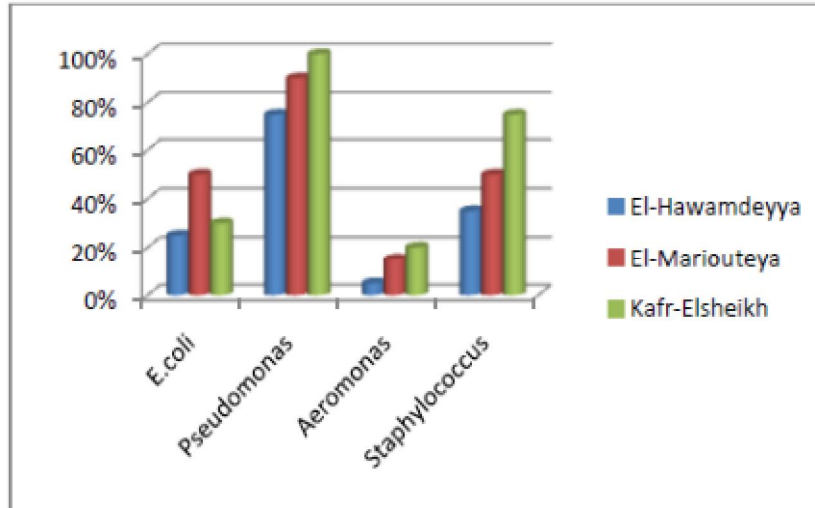


Fig (3) Incidence of the major food poisoning bacteria in examined Nile tilapia samples

Table (4) Incidence of the major food poisoning bacteria in examined Catfish samples

Locations	El-Hawamdeyya		El-Mariouteya		Kafr-Elsheikh	
	No	%	No	%	No	%
<i>E.coli</i>	5	25	10	50	6	30
<i>Pseudomonas aeruginosa</i>	15	75	18	90	20	100
<i>Aeromonas hydrophilla</i>	1	5	3	15	4	20
<i>Staphylococcus auerus</i>	7	35	10	50	15	75

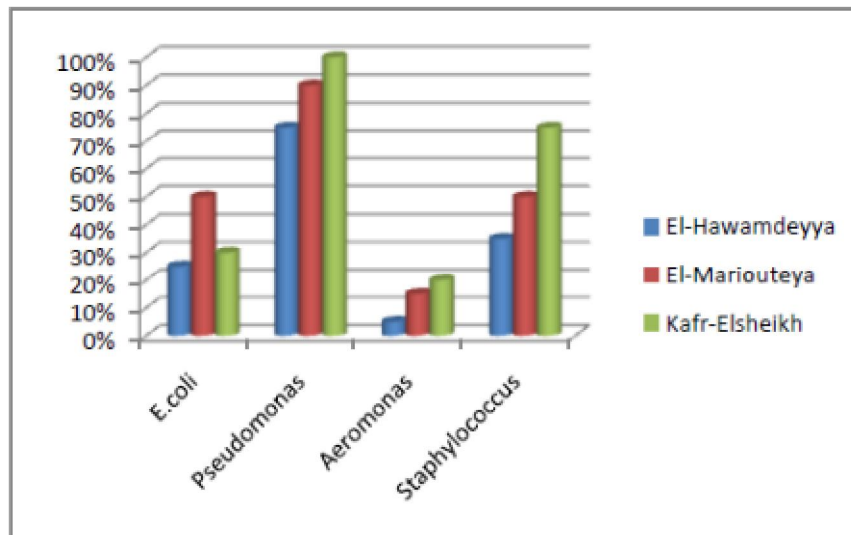


Fig (4) Incidence of the major food poisoning bacteria in examined Catfish samples

Table (5): Heavy metals per mg/kg in Nile tilapia and Catfish at different locations

Heavy metals	El-Hawamdeyya		El-Marioteya		Kafr-Elsheikh	
	Nile tilapia	Catfish	Nile tilapia	Catfish	Nile tilapia	Catfish
Lead	ND	0.22±0.15	0.3±0.1	0.5±0.1	0.2±0.08	0.23±0.15
Cadmium	ND	0.012±0.002	0.5±0.1	1.25±0.4	0.2±0.035	0.95±0.33
Iron	3±0.85	4±0.6	4.5±0.5	6.55±0.75	5.6±1.15	7.3±0.4
Copper	0.04±0.02	ND	0.9±0.07	1±0.3	0.5±0.2	0.8±0.18
Zinc	0.017±0.002	0.023±0.004	ND	ND	ND	ND
Chromium	1.5±0.3	0.4±0.2	1.9±0.33	1.9±0.1	0.75±0.1	0.95±0.13
Manganese	ND	ND	ND	ND	0.04±0.03	ND

Table (6) mean values of heavy metals before and after cooking in Nile tilapia from the three locations

Heavy metal	El-Hawamdeyya			El-Marioteya			Kafr-Elsikh		
	Raw	Fry	grill	Raw	Fry	Grill	Raw	Fry	Grill
Lead	ND ^a	0.01±0.007 ^a	ND ^a	0.3±0.1 ^a	0.43±0.15 ^a	0.17±0.07 ^a	0.2±0.08 ^a	0.1±0.03 ^a	0.09±0.05 ^a
Cadmium	ND	ND	ND	0.5±0.1 ^a	0.09±0.01 ^b	ND ^c	0.2±0.03 ^a	ND ^b	0.06±0.03 ^c
Iron	3±0.85 ^a	0.66±0.3 ^c	1±0.5 ^b	4.5±0.5 ^a	0.46±0.2 ^c	2.3±0.5 ^b	5.6±1.15 ^a	1.87±0.5 ^c	3.7±1 ^b
Copper	0.04±0.02 ^a	ND ^b	0.03±0.007 ^b	0.9±0.07 ^a	ND ^b	0.12±0.05 ^b	0.5±0.2 ^a	0.15±0.05 ^b	0.3±0.1 ^b
Zinc	0.017±0.002 ^a	ND ^a	0.003±0.002 ^a	ND	ND	ND	ND	ND	ND
Manganese	ND	ND	ND	ND	ND	ND	0.04±0.03 ^a	ND ^a	ND ^a
Chromium	1.5±0.3 ^a	0.65±0.3 ^c	1.2±0.35 ^b	1.9±0.33 ^a	1±0.3 ^c	1.4±0.25 ^b	0.75±0.1 ^a	0.07±0.01 ^c	0.46±0.09 ^b

Means in the same row having different superscripts are significantly different at P < 0.05

Table (7) mean values of heavy metals before and after cooking in Catfish from the three locations

Heavy metal	El-Hawamdeyya			El-Marioteya			Kafr-Elsikh		
	Raw	Fry	grill	Raw	Fry	Grill	Raw	Fry	Grill
Lead	0.22±0.15 ^a	0.4±0.2 ^a	0.1±0.07 ^{ab}	0.5±0.1 ^a	0.6±0.1 ^a	0.3±0.1 ^{ab}	0.23±0.15 ^a	0.3±0.1 ^a	0.08±0.05 ^a
Cadmium	0.012±0.002 ^a	ND ^b	ND ^b	1.25±0.4 ^a	0.5±0.2 ^b	0.9±0.4 ^b	0.95±0.33 ^a	0.46±0.15 ^b	0.7±0.25 ^b
Iron	4±0.6 ^a	0.9±0.1 ^b	1.9±0.2 ^c	6.55±0.75 ^a	1.9±0.5 ^b	4.2±0.8 ^c	7.3±0.4 ^a	3.2±0.45 ^b	4.4±0.6 ^c
Copper	ND	ND	ND	1±0.3 ^a	0.6±0.2 ^c	0.8±0.3 ^b	0.8±0.18 ^a	0.1±0.04 ^c	0.5±0.1 ^b
Zinc	0.023±0.004 ^a	0.003±0.002 ^a	0.005±0.002 ^a	ND	ND	ND	ND	ND	ND
Manganese	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chromium	0.4±0.2 ^a	0.13±0.05 ^b	0.16±0.1 ^a	1.9±0.1 ^a	0.5±0.15 ^b	1.6±0.3 ^a	0.95±0.13 ^a	0.35±0.1 ^b	0.4±0.1 ^a

Means in the same row having different superscripts are significantly different at P < 0.05

5. Conclusion

The microbiological examination of fish species from El-Marioteya and Kafr-Elsheikh contained elevated levels of coliform, fecal coliform and staphylococcus counts which may constitute a potential health risk for fish consumers.

Pseudomonas aeruginosa are highly isolated from all fish samples while, *Aeromonas hydrophilla* are the lowest.

Fish samples from El-Marioteya drainage canal were the highest in lead, cadmium, copper and chromium while fish samples obtained from Kafr-Elsheikh were the highest in iron and manganese but fish samples from El-Hawamdeyya was the highest in zinc only this attributed to the increase of heavy

metals in drainage water from the decomposition of the organic matter and /or the use of fertilizers and other chemicals in agriculture. More over fish samples collected from the River Nile (El-Hawamdeyya and Kafr-Elsheikh) accumulated a considerable concentrations but less than that recorded in fish collected from El-Marioteya drainage canal this could be attributed to the dilution of effluents discharged to the River Nile and its water current.

Among all types of fish cooking methods used in the study, frying showed a more pronounced effect on heavy metal content than grilling.

Further studies need to be performed on cooking methods at different conditions (i.e, time, temperature,

and cooking mediums) aimed to reduce the dangerous effects of heavy metals in fish meat.

Finally there is need for constant monitoring of heavy metals in fish, even when the exposure is low also monitoring of these trace metals is required in River Nile since the river serves as a source of drinking water, irrigation and fish for the local inhabitants in our study areas.

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5/28/2019