

1 Title: Assessing the chemical and microbiological quality of farmed tilapia in Egyptian fresh
2 fish markets

3 Running title: Quality of farmed tilapia in Egyptian fresh fish markets

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1 ABSTRACT

2 Fish make important contributions to food and nutrition security in low and middle income
3 countries; however, they are also prone to contamination with a range of chemical and
4 biological hazards. The presence of people's perception and health hazards has implications
5 for consumer acceptability and hence the potential contribution of fish to nutrition and health.
6 The aim of this study was to assess the chemical and microbiological quality of farmed tilapia
7 in Egypt. We conducted a systematic literature review resulting in 38 papers meeting
8 inclusion criteria. We also conducted a survey of seven hazardous chemicals in fish sampled
9 from farms (300 samples from 100 farms) and of 5 biological hazards as well as total
10 bacterial counts in fish sampled from retailers (300 samples from 100 retailers). The results
11 showed that the level of contamination with heavy metals and pesticides was lower than the
12 national and international permissible limits. On the other hand level of contamination of a
13 considerable proportion of samples with microbial pollutants was higher than the permissible
14 limits. Results from the literature indicated that, the level of contamination of wild tilapia was
15 higher than farmed tilapia, again in contradiction to common perceptions. Our results indicate
16 that the risk of human exposure to heavy metals and pesticides via consumption of farmed
17 tilapia is negligible compared to microbial hazards. These findings suggest that post-harvest
18 contamination is the major health risk in the tilapia fish value chain and we make
19 recommendations for addressing this.

20 Key words:

21 Farmed Tilapia; Heavy metals; Pesticide residues; Egypt

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1 1. INTRODUCTION

2 Globally, aquaculture accounts for about 50% of fish consumed and is one of the fastest
3 growing food sectors (Subasinghe et al., 2009) with a growth rate of approximately 6.2% in
4 2011 (FAO, 2013). In low and middle income countries, most farmed fish (>80%) is
5 produced in fresh water by small scale producers (Hastein et al., 2006). While fish is an
6 important source of low fat protein rich food and omega-three/omega-six fatty acids that
7 protect against adverse health effects, such as coronary heart disease and stroke (Domingo,
8 2007), there are also increasing concerns about foodborne hazards, chemical and microbial,
9 that might be present in fish. These concerns can also result in decreasing demand for farmed
10 fish (Smallwood and Blaylock 19991). This could negatively affect fish farmers and retailers
11 and decrease consumption and use of animal source food.

12 Egypt is the largest aquaculture producer in Africa and among the top 10 producer countries
13 worldwide; in 2015 the aquaculture production was 1.5 million tonnes¹. Published data on
14 public health hazards associated with farmed tilapia in the Egyptian fresh fish value chains
15 are scarce. Given the unprecedented production and consumption of tilapia in Egypt of over
16 half a million tonnes in 2011 (Macfadyen et al., 2012), it is critical to get an understanding of
17 potential contamination of this important food. A characterisation of farmed tilapia
18 production, marketing and consumption patterns in the Nile Delta identified various potential
19 points of potential contamination (Eltholth et al., 2015). Agriculture drainage canals, which
20 contain water that has been used for agricultural activities and constitute the main water
21 supply for most fish farms in this area were identified as sources of pesticide residues, runoff
22 derived fertilizers and metals that may contaminate farmed fish (Authman et al., 2013,
23 Authman et al., 2012, Authman, 2011, Authman and Abbas, 2007).

24
25 The aim of this study was to assess the chemical and microbiological quality of farmed tilapia
26 in the Nile Delta, Egypt. The objectives were 1) to measure chemical and biological
27 contamination levels at farm and retail levels, respectively and 2) to compare detected
28 contamination with values published in the scientific literature. Outcomes of this study are
29 important inputs for risk assessment and appraisal of strategies for management of human
30 exposure to chemical and/or microbiological health hazards via consumption of farmed
31 tilapia.

¹ <https://dailynewsegypt.com/2017/02/20>

2. MATERIALS AND METHODS

2.1. Study site and sampling

For the assessment of chemical pollutants, 300 whole tilapia of at least 100g were collected from 100 fish farms (three each) in Kafrelsheikh governorate, the main fish producing area in Egypt, in which about 55% of the farmed fish is produced (Macfadyen et al., 2011). Farms were selected randomly (Eltholth et al., 2015). Visits were scheduled with the owner, manager, or a worker who was authorised to talk to the enumerators. Upon visiting the farm, three fish from one pond, given that all ponds within the farm were connected, were collected in sterile plastic bags. Samples were transported to Kafrelsheikh University Central Laboratory of Environmental Studies (KUCLES) on ice in an ice box as soon as possible.

For the assessment of microbiological pollutants, tilapia samples were collected from 100 fresh fish retailers (20 wholesalers, 56 retailers and 24 street vendors) from Kafrelsheikh, Gharbia and Menoufia governorates in the Nile Delta. The target fish retailers were those serving the consumers in the study area; they were traced from consumers interviewed as described elsewhere (Eltholth et al., 2015). From each retailer three whole tilapia of at least 100g were taken from tilapia boxes offered for sale to customers. To make sure that tilapia were locally produced, retailers were asked about the source of tilapia. All samples were collected in sterile plastic bags and transported to the Central Diagnostic and Research Laboratory, Faculty of Veterinary Medicine Kafrelsheikh University on ice in an ice box.

2.2. Laboratory analysis

Samples were prepared and analysed for chemical and microbial contaminants according to the standard methods. Detailed description of the analysis for chemical and microbial contaminants is available in appendix 1.

2.3. Literature review

As consumers are more concerned about chemical pollutants of farmed tilapia and their perception about safety of farmed tilapia. We aimed to compare detected contamination with values published in the scientific literature, a systematic literature review was carried out. The following databases were searched: PubMed and Google Scholar. For the initial identification of primary studies the search terms “heavy metals”, “arsenic”, “lead”, “cadmium”, “mercury”, “muscles”, “pesticides”, “residues”, “organophosphorus” and “organochlorines” were applied in combination with “Egypt” AND “tilapia” using AND. The searches of Google Scholar were restricted to articles published since 2000 to December

1 2013. Titles and abstracts were screened for primary identification of relevant studies
2 according to the following criteria: (i) published in a scientific journal, (ii) in English
3 language and (iii) addressed at least one of the chemical pollutants under the scope of this
4 study. The following studies were excluded: review articles, experimental studies or trials for
5 assessing the impact of pollution on tilapia, and studies investigating the contamination of
6 fish species other than *Tilapia niloticus*. Additionally, studies investigating the contamination
7 of tilapia organs other than muscles were excluded, as only muscle tissue is commonly
8 consumed. Relevant articles were imported to the Endnote database, duplicates were removed
9 and the full text was obtained. Next, the articles were read in full and the concentration of
10 pollutants under the scope of this study was recorded. Simple descriptive statistics using
11 excel was used to summarize the findings of relevant studies.

12 2.4. Ethical approval

13 The present study received approval from the Ethics and Welfare Committee of the Royal
14 Veterinary College, London, UK (reference number URN 2012 1191) and Kafrelsheikh
15 University, Egypt. Oral informed consent was obtained from each study participant after
16 reading written consent form. The interviewers confirmed the participants' oral consent by
17 ticking the relevant boxes on the hardcopies. The consent form mainly explained about the
18 purpose of the study, the risks and benefits of participation in the study, conditions of
19 confidentiality and the right to refusal or withdrawal from the study.

20 3. RESULTS

21 3.1. Chemical analysis of tilapia samples from fish farms

22 Six pesticides and one heavy metal were detected (aldrin, dieldrin, endrin, heptachlor,
23 heptachlor-epoxide (beta) and lindane pesticides and mercury respectively). All pesticide
24 results were well within the maximum permissible limits (MPL) defined by Codex
25 Alimentarius Commission (CAC, 2009). The concentration of mercury in all contaminated
26 samples was lower than the MPL (0.50 ppm) stated by Egyptian Organization for
27 Standardization and Quality (EOS, 2010). Arsenic, lead and cadmium were not detected in
28 any of the samples. Results for the chemical hazards in farmed tilapia are in table 1.

29 Table 1: The concentrations of pesticide residues in tilapia from fish farms in Kafrelsheikh governorate, Egypt

Concentration (ppb*)	Pesticides					
	Aldrin	Dieldrin	Endrin	Heptachlor	Heptachlor-	Lindane

	Epoxide					
Minimum	7.48	9.2	0.34	0.8	0.34	1.2
Maximum	35.42	33.6	12.4	6.96	2.91	5.9
Mean	19.18	16.78	2.37	2.68	1.29	3.04
Standard deviation	7.64	6.18	2.69	1.48	0.56	1.33
MPL**	300	300	300	200	200	200

1 *ppb=part per billion, **MPL= Maximum permissible limits adapted from (Yahia and
2 Elsharkawy, 2014)

3 3.2. Bacteriological analysis of fish samples from retail sale

4 The mean total counts of aerobic plate count (APC), *E. coli*, *S. aureus* and *V.*
5 *parahaemolyticus* in positive samples are listed in table 2. The proportions of samples with
6 count higher than the MPL stated by EOS (EOS 2005) were 13.7%, 8.0%, 7.7%, 3.3%, 13%
7 and 12.3% for APC, *E. coli*, *L. monocytogenes*, *Salmonella*, *S. aureus* and *V.*
8 *parahaemolyticus*, respectively. Out of the tested samples, only 64.3% complied with all
9 bacteriological standards: the level of APC and tested pathogens were under the MPL stated
10 by the EOS. The mean APC (6.2×10^5 cfu/g) for samples collected from the whole sale market
11 was lower than that from the retail markets and the latter was lower than that from street
12 vendors.

13 Table 2: Bacteriological characterisation of fresh tilapia samples collected from retail sale in the Nile Delta of Egypt

Foodborne pathogens	Bacterial count		Egyptian standard cfu/g*	Samples > Egyptian standards (%)
	Mean	SD		
Total aerobic count (10^5 cfu/g)	6.2	3.80	1×10^6	13.7
<i>E. coli</i> (10^3 cfu/g)	0.73	0.54	100	8.0
<i>L. monocytogenes</i> **	NA	NA	0.0	7.7
<i>Salmonella</i> spp.**	NA	NA	0.0	3.3
<i>S. aureus</i> (10^3 cfu/g)	1.17	1.03	1000	13.0
<i>V. parahaemolyticus</i> (10^3 cfu/g)	0.89	0.91	0.0	12.3

14 *MPL stated by EOS (EOS, 2005) for fresh chilled fish, ** The count for *L. monocytogenes*
15 and *Salmonella* spp. Was not presented as enrichment steps were used.

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3.3.Literature review for chemical pollutants in tilapia

The initial search revealed 6108 publications. After exclusion of duplications and applying the inclusion criteria, 38 papers were retained. From these articles data were extracted and summarised in appendix 2 and 3 for pesticide residues and heavy metals, respectively. Not all data from relevant papers were extracted, only data concerned with the pollutants under the scope of this study. Of the studies included, 8.7% and 14.3% were concerned with the detection of pesticide residues and heavy metals in cultured fish, respectively. The majority of relevant studies were concerned with contamination of water and wild tilapia in Egyptian lakes and the level of pollution in the Nile tributaries and agricultural draining canals. The level of pesticide residues reported in all studies was lower than the MPL, Table 3. The concentration of heavy metals was higher than the MPL in most of cases. Results from these studies suggest that the level of contamination of wild tilapia, particularly from lakes and drainage canals, was higher than that of farmed tilapia. Also the average level of contamination, particularly with heavy metals, was higher than that detected in the current study.

Table 3: Summary of the results from scientific literature for heavy metals in tilapia muscles collected from different sources in Egypt.

Concentration (ppm)	Lead	Cadmium	Mercury
Minimum	0.002	0.01	0.01
Maximum	48.7	10.85	67.10
Mean	4.49	1.12	8.69
SD	8.09	2.32	19.75
MPL*	0.30	0.05	0.50

MPL= maximum permissible limits for heavy metals in fish stated by Egyptian Organization for Standardization and Quality (EOS, 2010).

4. DISCUSSION

This study assessed the prevalence and level of contamination with selected chemical and microbiological pollutants in farmed tilapia produced and marketed as fresh fish in the Nile delta of Egypt. The results of this study showed that the level of contamination of farmed tilapia, in the Nile delta, with pesticide residues and heavy metals was within the MPL. However the level of contamination with certain microbiological contaminants was higher than the MPL which could be due to post-harvesting contamination.

4.1. Chemical pollutants in farmed tilapia

Six pesticide residues were detected and the level was within the MPL according to CAC (CAC, 2009). According to the Egyptian standards “*the maximum levels should be set at a strict level which is reasonably achievable by following good agricultural, fishery and manufacturing practices and taking into account the risk related to the consumption of the food*” (EOS, 2010). In general, the level of detected chemical pollutants was lower than that from previous studies particularly for tilapia samples collected from lakes and river Nile tributaries. These results contradict the perception of many consumers that wild caught tilapia are safer than farmed one (Eltholth et al., 2015). The low level of heavy metals in tilapia tissues in this study may be due to the frequent changing of water in fish ponds and the use of branded fish feed by more than 90% of fish farmers (Eltholth et al., 2015). Apart from water sources, management, type of fish feed and feeding system may influence the level of contamination of fish tissues with metals and/or pesticide residues (Trocino et al., 2012). The metal concentration in different parts of tilapia varies according to the metabolic activities of the organs and the routes of exposure. Its concentration in muscle tissues is lower than other organs (Authman et al., 2012, Authman et al., 2013). This variation of concentration and distribution of chemical pollutants indicate that the risk of human exposure to chemical pollutants via consumption of tilapia will depend on the way of processing, cooking and consumption behaviour. The main threats to human health from heavy metals are from lead, cadmium, mercury and arsenic (Järup, 2003). Serious health effects on both adults and children such as cancer and damage to the nervous system have been associated with the consumption of fish contaminated with these heavy metals (Castro-González and Méndez-Armenta, 2008, Vieira et al., 2011). However, these effects are typically seen upon consumption of fish contaminated at much higher levels, than those detected in this study where no heavy metal exceeded MPL. Variations in the concentrations of chemical pollutants

in farmed and wild tilapia may be associated with the agricultural cycle in Egypt and the use of fertilizers and pesticides.

4.2. Bacteriological pollutants in farmed tilapia

The mean APC for more than 85% of samples was within the MPL defined by Egyptian standards (10^6 cfu/g) for marketed fresh fish for all samples (EOS, 2005). Pre and post-harvesting contamination and handling of tilapia were identified as potential sources of contamination (Eltholth et al., 2015). Methods of harvesting and handling of tilapia from the farm to retail sale could contribute to the bacterial load of tilapia given the lack of a proper cold chain. This impact will be more during summer season where ambient temperature would be higher than 30 °C. Fish could be contaminated post-capture from fishing tools, contaminated water, contaminated ice, soiled surfaces and boxes, as well as by poor hygienic handling practices (Mhango et al., 2010). Environmental factors such as ambient temperature and relative humidity may play a role in the contamination of tilapia and the level of contamination could vary seasonally. Studies reported that, Gram-positive bacteria such as *Staphylococcus spp.* were increased in sea foods during storage at 2–4°C (Rantsiou et al., 2005, Nimrat et al., 2006). While storing fish at 0°C as soon as possible after capturing will maintain the quality of fish by delaying the spoilage process (Farak, 2012). Microbial pollutants would increase the spoilage of fish and consequently decreasing the shelf life. Bacterial pollutants not only affect the quality of tilapia but also may posing food safety problems.

Very few samples were contaminated with *E. coli*, *L. monocytogenes*, *Salmonella spp.*, *S. aureus* and *V. parahaemolyticus*. Results of the bacteriological analysis were better than that from previous studies (Emire and Gebremariam, 2010, Hamed et al., 2013). *Salmonella spp.* was detected in 10% of seafood samples collected from markets in Alexandria, Egypt (Bakr et al., 2011) which has similar or even lower ambient temperature than the area for the current study. In fish markets with similar environmental conditions the prevalence of *Staphylococcus spp.* in whole and gutted tilapia samples from street vendors in Gaborone, Botswana was 62% and 41%, respectively (Mhango et al., 2010). *Vibrio spp.* was detected in 52% of seafood samples collected from markets in Alexandria, Egypt (Bakr et al., 2011, Kaysner and DePaola Jr, 2000). As *V. parahaemolyticus* in outbreak investigations linked with sea food consumptions. The presence of pathogenic bacteria should be of concern to tilapia handlers, both on retail and households, and consumers but this would depend on the way of processing, cooking and consumption behaviour.

Although, our findings and data from literature indicated that farmed tilapia is safer than wild caught fish, consumers' perceived wild caught fish as safer and pay more for it. This might be due to fears that farmed tilapia are fed on poultry manure and dead poultry. Also some retailers claiming that, they are selling good quality fish from natural resources. The lack of knowledge and traceability affect consumers' behaviour and consequently has negative impacts on the farmed tilapia value chain (Eltholth et al., 2015). Farmed fish can be of good quality but for value chain to develop need to a) **manage hazards particularly post-harvest contamination**; b) improve trust; and c) better align hazard perception with reality.

5. CONCLUSIONS

The results of this study show a relatively low level of chemical contaminants but a higher level of biological contaminants **in farmed tilapia in the Nile Delta**, suggesting the priority is reduction in microbial hazards. This could be supported by promotion of best management practices and use of hazard analysis critical control points (HACCP). Microbiological criteria can be used for assessing the HACCP and other hygienic measures along the farmed tilapia production chain. Further information on consumption amounts and the impact of processing and cooking on hazards is needed in order to estimate the risk to human health: however this initial study suggests farmed tilapia does not present a major or exceptional risk to human health. It also indicated that, consumers' perception **in the study area** is poorly aligned with reality.

6. ACKNOWLEDGEMENT

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Appendix 1 laboratory analysis

1. Chemical analysis

At the Kafrelsheikh University Central Laboratory of Environmental Studies (KUCLES), after cleaning the tilapia and removal of scales, two separate tissue samples of 10 g each were taken from each fish from the dorsal muscles. One sample (10 g) was for the detection of heavy metals and the other for pesticide residues. Samples were immediately frozen at -20°C until analysis. The following protocols were used for analysis: For the detection of organophosphorus (OP) and organochloride (OC) residues a protocol as described by Darko et al. (2008) was adopted. Each sample was ground in a blender to obtain a homogenous composite. Pesticide residues were extracted using liquid extraction with 150 mL of acetone and n-hexane (20:80 v/v). The tissue sample was placed into a beaker containing 50 mL from the solvent mixture and was shaken for 20 min, followed by filtration. This process was repeated twice, each time with 50 mL of the solvent mixture. The collected filtrate then was passed through anhydrous sodium sulfate. The extracts were concentrated to 1 mL using vacuum rotary evaporator. The extract for each sample was then dissolved in hexane (10 mL) and passed through pre-conditioned octadecyl C-18 columns at a rate of 2 ml/min to clean up. The column was washed with 30% methanol (1 mL) followed by ultrapure water (1 mL) and then allowed to dry. The trapped sample (analyte) in the column was eluted five times with 0.5 mL aliquots of hexane to recover the pesticides residues. Hexane in the sample was then evaporated off leaving the residues alone in the vial. The dried sample was dissolved in hexane (1 mL), thoroughly mixed and then transferred to auto sampler vials ready for gas chromatography. The OC and OP residues were analyzed using Gas chromatography–mass spectrometry (GC-MS, THERMO SCIENTIFIC). All chemicals used were HPLC grade.

For the detection of arsenic, lead and cadmium, fish samples were dried at 65°C for 48 h in a desiccator. One gram of each sample was digested using dry ashing method in a muffle furnace at 450° C for 5h, then the ash was extracted using 20% hydrochloric acid as described in detail elsewhere (Jones Jr et al., 1991). All heavy metals were measured by atomic absorption spectrometry (Unicam 969, Analytical Technology Inc., Cambridge, United Kingdom).

Mercury was detected directly from tilapia tissue samples using NIC Mercury Analyzer MA-3000. The following standard solutions were prepared and used: 1) L-cysteine 100mg/L: 100 mg of L-cysteine HSC₂CH(NH₂)COOH was added to a 1000 ml flask with water and 2 ml of nitric acid was then added to make a total of 1000 ml of solution. The flask was then

shaken to stir the solution and stored in a cool dark place, 2) Mercury standard solution 10 mg/L: 1 ml of mercury standard solution for atomic absorption (1000 ppm) was to add L-cysteine solution (100 mg/L) to make a total of 100 ml and 3) Mercury standard solution 0.1mg/L: L-cysteine (100 mg/L) was added to 1ml of the previous prepared solution to make a total of 100 ml. The manufacturer's protocol was followed for preparing the standard solutions and the measurement methods.

2. Bacteriological analysis

Samples were prepared for bacterial isolation and enumeration by cutting out, with sterile instruments, 25 g of dorsal muscles. Samples were then homogenized in 225 ml of sterile buffered peptone water. The homogenate samples were inoculated on standard plate count media and media for the selective isolation of bacteria. The isolated colonies were subjected to bacteriological characterization procedures. First, selective media were used for the identification of potential isolated colonies, while further identification was conducted by using morphological and biochemical tests. Aerobic plate count, *Salmonella* spp., *E. coli*, *S. aureus*, *L. monocytogenes*, and *V. parahaemolyticus* tests were performed as described below. All media used were from Oxoid Ltd, UK. For the APC, homogenised samples were serially diluted and plated using the pour-overlay method on solid medium using standard plate count agar. Petri dishes were then incubated in inverted position for 48 ± 2 h at 37°C. (Maturin and Peeler, 2001) Presumptive *E.coli* was enumerated on eosin methylene blue (EMB) agar. (Feng et al., 2002) After incubation, typical colonies with metallic sheen were counted after which, 3-5 colonies were selected for biochemical confirmation. *Listeria monocytogenes* was enriched on Fraser Broth at 35°C for up to 48 h and then inoculated on PALCAM *Listeria* agar at 37°C for up to 48 h. (Hitchins and Jinneman, 2003) *Salmonella* spp. were detected by a pre-enrichment with Rappaport-Vassiliadis peptone broth at 42°C for 24 h, then plating on xylose lysine deoxycholate (XLD) agar at 42°C for 24 h (ISO 6579, 2002). For the detection of *V. parahaemolyticus*, alkaline peptone water (APW) was used as a diluent and thiosulphate citrate bile salt (TCBS) agar 3% for 24 h at 37°C as selective media (Sankar et al., 2012). Baird Parker agar (BPA) was used for the detection and enumeration of *S. aureus*, incubated at 37°C for 24–48 hours (Vanderzant and Splittstoesser, 1992). For biochemical characterisation of isolates, the following biochemical tests were used: catalase, oxidase, sugar fermenter, indol production, methyl red, Voges-Proskauer, Simmon's citrate, urease, blood agar and TSI (Cheesbrough, 1987, Holt et al., 1994).

Appendix 2: The concentrations of pesticide residues in tilapia muscle tissues from different sources in Egypt (ND= not detected, NM= not measured)

Source of tilapia	Pesticide residues (Mean concentration)							Reference
	Unit	Aldrin	Dieldrin	Endrin	Heptachlor	Heptachlor-Epoxy (beta)	Lindane	
Fish markets (Fresh)	ng/g	ND	1.69	1.89	1.49	ND	0.58	(Ahmed and El-Saad, 2010)
Fish markets in (Grilled)	ng/g	ND	0.66	0.71	0.85	ND	ND	
Assiut_Elwasta	µg/kg	0.61	ND	NM	NM	NM	0.89	(Yahia and Elsharkawy,
Assiut_Mankbad	µg/kg	0.22	ND	NM	0.18	NM	0.47	2014)
Lake Manzala	ng/g	NM	NM	NM	NM	NM	0.91	(Abbassy et al., 2003)
Lake Manzala	µg/kg	0.033	NM	0.026	0.003	0.059	NM	(Azab et al., 2013)
Lake Manzala	µg/kg	8.6	NM	NM	1.35	NM	2.508	(Abou-Arab et al., 1995)
River Nile	µg/kg	3.85	NM	NM	2.63	NM	2.2	
River Nile	µg/kg	ND	ND	23.94	ND	ND	8.93	(Aly and Badawy, 1984)
Fish Farms	ng/g	ND	ND	NM	NM	NM	1.054	(El-Mekkawi et al., 2009)
Fish Farms	ng/g	13.038	10.125	NM	NM	NM	9.5	
Lake Manzala	ng/g	ND	ND	ND	0.14	ND	1.49	(Yamashita et al., 2000)
River Nile	ng/g	ND	ND	ND	0.10	ND	0.18	
Lake Idku	ng/g	0.023	0.130	0.028	NM	NM	0.025	(Abdallah and Morsy, 2013)
Lake Idku	µg/kg	ND	ND	<0.15	ND	ND	4.83	(Badawy and El-Dib, 1984)
Lake Maryut	µg/kg	ND	ND	<0.15	ND	ND	4.53	
Fresh water canals	ng/g	0.802	1.423	1.47	0.59	0.639	0.914	(Nasr et al., 2009)
Drain water canals	ng/g	0.614	1.597	1.75	2.576	1.786	2.01	
Lake Qarun	mg/kg	0.004	ND	ND	ND	ND	0.008	(Mansour and Sidky, 2003)

Appendix 3: The concentrations of heavy metals in tilapia muscle tissues from different sources in Egypt (ND= not detected, NM= not measured)

Source of tilapia	Heavy metals (Mean concentration)				Reference
	Unit	Cadmium	Lead	Mercury	
Illegal fish farm in Sabal drainage canal	mg/kg	4.35	3.02	2.35	(Authman et al., 2012)
Fish farms	mg/kg	1.21	1.52	3.50	(Kaoud and El-Dahshan, 2010)
Fish farms in Kafrelsheikh	µg/kg	2.33	6.27	NM	(Abumourad et al., 2013)
Fish farms in AL-Abasa	µg/kg	2.27	18.8	NM	
Fish farms in EL-Fayoum	µg/kg	4.27	8.63	NM	
Suez fish farm	mg/kg	NM	1.65	NM	(Shereif and Mancy, 1995)
Fish farm in Lake Manzala	mg/kg	NM	11.25	NM	
Fish ponds with water supply from Bahr El-Baquar drain	mg/kg	0.317	0.42	0.02	(HASSANIN, 2008)
Shanawan drainage canal, Al-Minufiya	mg/kg	5.30	48.70	67.1	(Khallaf et al., 1998)
Private fish farm at Fayoum Governorate	mg/kg	0.10	6.38	NM	(Mansour and Sidky, 2002) 1997/1998
Lake Qarun	mg/kg	0.16	1.65	NM	
Private fish farm at Fayoum Governorate	mg/kg	0.01	0.01	NM	(Mansour and Sidky, 2002) 1998/1999
Lake Qarun	mg/kg	0.08	0.06	NM	
El-Shoura fish farm in El-Fayoum Governorate	mg/kg	2.20	6.50	NM	(Ali and Abdel-Satar, 2005)
Sabal drainage canal, Al-Minufiya Province	mg/kg	3.40	31.95	39.13	(Authman, 2008)
Lake Edku	µg/g	ND	0.24	NM	(Abdallah and Morsy, 2013)
Lake Edku	µg/g	0.19	0.59	NM	(Saeed et al., 2008)
Lake Borollus	µg/g	0.01	0.05	NM	
Lake Manzala	µg/g	10.36	10.1	NM	
Lake Edku	µg/g	0.21	0.68	NM	(Sassd, 2011)
Lake Edku	µg/g	0.27	0.52	NM	(Saeed, 2013)
Lake Brullus	mg/kg	NM	0.30	NM	(Zaghloul et al., 2007)
Lake Brullus	mg/kg	ND	10.75	0.13	(Ahmed, 2013)

Bahar tera	mg/kg	ND	9.60	0.03	
River Nile at Monofya governorate	mg/kg	0.22	1.03	0.33	(El-Kattan and Nahla, 2008)
Ismalia canal	mg/kg	0.01	0.19	NM	(Elghobashy et al., 2001)
River Nile (Shubra El-Khicma, Cairo sector)	mg/kg	0.04	1.22	NM	
Maazala Lake	mg/kg	0.35	3.2	NM	
Lake Edku	mg/kg	0.11	1.57	NM	
Lake Borollus	mg/kg	0.03	0.22	NM	
Lake Qarun	mg/kg	0.01	0.33	NM	
Lake Maryut	mg/kg	0.53	1.12	NM	
Lake Maryut	mg/kg	0.24	2.77	NM	(Arafa and Ali, 2008)
Fish market survey	mg/kg	0.01	0.06	0.02	(Khorshed, 2009)
River Nile/Upper Egypt	mg/kg	NM	14.16	NM	(Yacoub and Gad, 2012)
River Nile	mg/kg	0.79	5.82	0.01	(Osman, 2012)
Fish markets survey	µg/kg	0.98	0.13	4.88	(Essa and Rateb, 2011)
Sabal draiage ncanal (Al-Menoufiya Province)	mg/kg	10.85	10.21	8.66	(Authman et al., 2013)
Lake Manzalah	µg/g	1.33	5.36	NM	(Abdel-Satar and Geneid, 2009)
Lake Manzalah	mg/kg	0.64	3.37	NM	(Abdel-Satar and Yacoub, 2005)
Elsalam canal	mg/kg	0.66	3.48	NM	
Lake Edku	mg/kg	0.27	0.52	NM	(Saeed, 2013)
Abu Za'baal ponds	µg/g	3.60	21.30	NM	(Abdo, 2006)
Lake Nasser	µg/g	1.25	1.75	NM	(Mohamed, 2008)
Sharkia province	µg/g	0.81	3.34	NM	(El-Sayed et al., 2011)
Lake Manzala	µg/g	0.11	3.81	NM	(Hamed et al., 2013)
High Dam Lake, Aswan	mg/kg	0.02	0.13	NM	(Rashed, 2001)
Lake Qarun	µg/g	1.23	6.72	NM	(Fishar and Ali, 2005)
Lake Qarun	mg/kg	0.06	0.09	NM	(Mansour and Sidky, 2003)
Lake Qarun	mg/kg	NM	3.76	NM	(Omar et al., 2013)
Qarun fish farms	mg/kg	NM	2.38	NM	(Omar et al., 2013)
Wadi El-Rayan protected area lakes	mg/kg	0.11	0.157	0.01	(Abdou, 2009)

Farmed tilapia	mg/kg	NM	2.41	NM	(Gabr and Gab-Alla, 2007)
Wild tilapia	mg/kg	NM	2.32	NM	(Gabr and Gab-Alla, 2007)
Ponds with irrigation water	μg/g	0.13	1.54	NM	(El-Nemaki et al., 2008)
Ponds with agriculture drainage water	μg/g	0.04	0.90	NM	(El-Nemaki et al., 2008)
River Nile tributaries	μg/g	0.76	1.51	NM	(Malhat, 2011)
River Nile-Rosetta branch	mg/kg	1.51	5.94	NM	(Yehia and Sebaee, 2012)
River Nile-Damietta Branch	mg/kg	NM	14.65	NM	(Gad and Toufeek, 2010)
Fish markets/Sharkiah governorate	mg/kg	0.02	0.15	0.37	(El-Barbari and Sami, 2012)
Fish markets	mg/kg	NM	0.52	NM	(Salim et al., 2003).
