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FULL LENGTH ARTICLE

Chemical compositions and heavy metal contents of *Oreochromis niloticus* from the main irrigated canals (rayahs) of Nile Delta

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Proximate composition;
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TVB-N

Abstract The present study aimed to assess the seasonal variations of the proximate chemical composition, physicochemical, microbiological aspects and heavy metal concentrations of *Oreochromis niloticus* muscles collected from The Nile rayahs from spring 2014 to winter 2015. Rayahs are the main irrigated canals of Nile Delta, Egypt and represent El Tawfiki, El Menoufy, El Behery, and El Nasery rayahs. Results showed a spatial and temporal significant difference ($p < 0.01$) in the proximate composition and Physicochemical aspects of *O. niloticus* muscles. The moisture, protein, fat, ash, carbohydrates and calorific values varied between (78.55–80.77%), (16.10–17.88%), (1.10–1.95%), (0.55–1.50%), (0.10–0.94%) and (78.37–89.73%), respectively. Heavy metal accumulation in the *O. niloticus* muscles showed irregular distributions with descending order of: Fe > Zn > Mn > Cu > Pb > Cd. Generally, heavy metals, TVB-N, TMA, TBA and TVC did not exceed the maximum permissible limits in the tissues of *O. niloticus*. The values of Hazard Index (HI) and Hazard Quotient (HQ) are lower than the acceptable limits, which indicate that the metals in *O. niloticus* in the Nile rayahs, Egypt, do not pose any particular human health risk concern. Therefore, *O. niloticus* muscles collected from four rayahs are safe for human consumption and could be used as a source of healthy diet for humans.

Introduction

Egypt is the 8th global aquaculture producer and is the largest in Africa. In 2011 the aquaculture production was about 986,820 tons (FAO, 2013). According to the General Authority for Fisheries Resource Development (GAFRD), a significant contribution to income, food security and employment is created by the aquaculture sector. It is also a rapidly growing sector: fish consumption in Egypt rose from 8.5 kg/person/year during 1996 to 15.4 kg during 2008 and 20.8 kg during 2013 (Macfadyen et al., 2012; FAO, 2014 and Eltholth et al., 2015).

Tilapia is the most important aquatic species produced and consumed in Egypt. Tilapia production has grown over the

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years; in 2010 it reached 687,400 Mt valued at US$991 million (L.E. 5478.4 million) representing 38% of the total fish value and 53% of total fish production (GAFR, 2011). Body composition of the same fish species may vary depending on changes in the environmental conditions. Water pollution is the most important factor affecting the quality of fish production in its natural habitats (Younis et al., 2014).

Chemical composition varies according to season, environment, sexual cycle, maturity stage, feed, age, organs and also muscle location. In addition, mineral concentrations of fish muscle zones may be influenced by different biological factors. Consequently, it is very important to determine the seasonal biochemical composition of fish in order to ensure its quality (Khitouni et al., 2010; Kozlova, 1997; Gockse et al., 2004; Zlatanos and Laskaridis, 2007; Noël et al., 2011; Roy and Lall, 2006). Evaluation of proximate composition is necessary to indicate that, fish tissues have healthy safe qualities and meet the national and international standard specifications (WHO/FAO, 2011).

Tilapia is an important food resource for human consumption in Egypt. Thus, this study aims to assess the effects of seasonal variations on the proximate chemical composition, physicochemical aspects and heavy metal concentrations in the muscles of *Oreochromis niloticus* that were collected from El Tawfiki, El Menoufy, El Behery and El Nasery rayah, River Nile Egypt (Fig. 1) from spring 2014 to winter 2015.

Materials and methods

Fish samples collection and preparation

Nile tilapia (*O. niloticus*) fish samples were collected seasonally from the four rayahs from spring 2014 to winter 2015 (10 fish were taken randomly from each one). This species was selected on the basis of its dominance and importance to local human fish consumption. Fish samples were thoroughly washed with tap water to remove any adhering contaminants and then transported using an icebox to the pollution laboratory, National Institute of Oceanography and Fisheries. Mean weights and lengths of investigated fish samples were (350 ± 57.73 g) and (25.5 ± 1.32 cm), respectively. Upon arrival fish samples were re-washed thoroughly with potable water then beheaded, skinned and dissected (using plastic tools) to obtain muscles, gills and bones. Chemical composition, physicochemical characteristics and heavy metals concentrations were determined in the fish muscles only.

Analytical methods

Proximate compositions (moisture, protein, fat and ash contents) and trimethylamine nitrogen (TMA-N) were determined according to (A.O.A.C, 2002). Total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) and pH value were estimated as described by (Pearson, 1991). Ten grams of fish samples was diluted in 90 ml of peptone water and homogenized mechanically for 1 min. Sequential dilutions in peptone water (0.1% w/v) were made, 0.1 ml inoculums were plated out using plate count agar (SPCA, Oxoid) to determine the (TBC) expressed as (cfu g^{-1}) fish muscle and the plates were incubated for 48 h at 37 °C (FAO, 1992).

For heavy metal analysis 0.5 g of dried fish muscles was placed into PYREX Erlenmeyer flasks (and then 10 ml 2:1 v/v concentrated nitric acid and perchloric acid mix was add). The digestion was done on a hotplate (200–250 °C) until the solutions were clear. The solutions were then filtered through an acid resistant 0.45 mm filter paper and made up to 50 ml each with distilled water (Kotze et al., 2006). The samples were stored in clean plastic bottles prior to the determination of Fe, Zn, Cu, Mn, Pb and Cd concentration using an atomic absorption reader (SavantAAS with GF 5000 Graphite Furnace) and the results were given as mg/kg of dry weight and then converted to ppm wet weight basis.

Figure 1 Map of the study area (cited from Goher, 2015).

Statistical analysis

A two-way analysis of variance ANOVA analysis, followed by a post-hoc comparison using Tukey's test, were computed by Excel-Stat software to identify significant differences. Pearson's correlation analysis was achieved to estimate the possible relationships among the different variables.

Results and discussion

Proximate chemical composition of Nile tilapia

Variations of moisture, protein, fat, ash and carbohydrates (% of fresh fish: mean values ± SD) and energetic values (in kcal/100 g fresh fish ± SD) of *O. niloticus* collected from The Nile rayahs during the study period are illustrated in (Table 1).

The present study shows that the moisture varied in the ranges of (78.78–80.39)%, (78.79–80.77)%, (78.55–80.19)% and (78.66–80.26)% in of *O. Niloticus* muscles from El Tawfiki, El Menoufy, El Behery, and El Nasery rayahs, respectively, while the protein contents changed between (16.97–17.86)%, (16.62–17.64)%, (16.10–17.70)% and (16.95–17.88)%, respectively. On the other side the fat contents in the mussels of *O. Niloticus* from El Tawfiki, El Menoufy, El Behery, and El Nasery rayahs, fluctuated between (1.30–1.79)%, (1.10–1.58)%, (1.15–1.85)% and (1.21–1.95)%, respectively. Carbohydrates contents were found in the ranges of (0.15–0.68%)%, (0.16–0.94)%, (0.17–0.72)% and (0.10–0.30)% respectively. In the same manner the ash contents were recorded in the ranges of (0.55–1.19)%, (1.1–1.33)%, (1.08–1.50)% and (1.21–1.95)%, respectively. Similar to the above results energetic values changed between (80.18–89.23), (78.55–80.19), (80.76–89.73) and (80.29–89.20) kcal/100 g, respectively.

It must be noted that the highest values –based on seasonal average – of fat (1.74%), ash (1.35%) and energetic values (85.98 kcal/100 g) were recorded in the mussels of *O. Niloticus* caught from El-Rayah El-Nasery. while El-Rayah El-Menoufy showed higher values of moisture (79.89%) and carbohydrates (0.57%). The results showed that El-Rayah El-Tawfiki recoded higher values of protein (17.4%). It was also noted that, the highest values of moisture in the edible part of *O niloticus* were recorded during spring and summer, while higher values of protein, fat and calorific values were recorded during autumn and winter.

The obtained results agree with those reported by Love (1980), Javaid et al. (1992), Oliveira et al. (2003), Ibrahim et al. (2008), Saeed (2013) and Younis et al. (2014) who each reported that, the body composition of the same fish may change in relation to differences in water quality, feeding conditions, sex, maturity state, environmental conditions and the period during which the fish was captured.

Physicochemical and microbiological aspects

The freshness of fish is the most fundamental important criterion for evaluating the quality of fishery products and fish (Rodríguez-Jérez et al., 2000). The seasonal variations on physicochemical quality aspects (on wet weight basis) in muscle tissue of *O. niloticus* collected from four rayahs are presented in (Table 2).

Table 1 Variations of moisture, protein, fat, ash and carbohydrates (%) ± SD and energetic values (in kcal/100 g fresh fish ± SD) of *Oreochromis niloticus* collected from the Nile rayahs.

Seasons	Moisture	Protein	Fat	Ash	Carbohydrates	Energetic values
El-Rayah El- Tawfiki						
Spring	80.39 ± 0.45	16.97 ± 0.03	1.30 ± 0.02	1.19 ± 0.01	0.15 ± 0.05	80.18 ± 0.10
Summer	80.09 ± 0.31	17.15 ± 0.05	1.70 ± 0.05	0.55 ± 0.02	0.51 ± 0.01	85.94 ± 0.05
Autumn	79.50 ± 0.50	17.86 ± 0.05	1.45 ± 0.01	0.99 ± 0.03	0.20 ± 0.03	85.29 ± 0.17
Winter	78.78 ± 0.81	17.60 ± 0.02	1.79 ± 0.01	1.15 ± 0.03	0.68 ± 0.03	89.23 ± 0.03
Average	79.69 ± 0.52	17.40 ± 0.04	1.56 ± 0.02	0.97 ± 0.02	0.39 ± 0.03	85.16 ± 0.09
El-Rayah El-Menoufy						
Spring	80.77 ± 0.50	16.62 ± 0.10	1.17 ± 0.03	1.10 ± 0.03	0.34 ± 0.01	78.37 ± 0.08
Summer	80.46 ± 0.49	16.75 ± 0.05	1.30 ± 0.05	1.33 ± 0.02	0.16 ± 0.01	79.34 ± 0.05
Autumn	79.53 ± 0.28	17.11 ± 0.09	1.10 ± 0.03	1.32 ± 0.05	0.94 ± 0.04	82.10 ± 0.10
Winter	78.79 ± 1.10	17.64 ± 0.10	1.58 ± 0.08	1.16 ± 0.01	0.83 ± 0.03	88.10 ± 0.10
Average	79.89 ± 0.59	17.03 ± 0.09	1.29 ± 0.05	1.23 ± 0.03	0.57 ± 0.02	81.98 ± 0.08
El-Rayah El-Behery						
Spring	80.19 ± 1.01	17.41 ± 0.10	1.15 ± 0.01	1.08 ± 0.06	0.17 ± 0.02	80.67 ± 0.08
Summer	80.16 ± 0.75	16.10 ± 0.10	1.56 ± 0.05	1.50 ± 0.03	0.68 ± 0.03	81.16 ± 0.03
Autumn	78.55 ± 0.50	17.70 ± 0.25	1.85 ± 0.01	1.33 ± 0.04	0.57 ± 0.05	89.73 ± 0.05
Winter	78.89 ± 0.55	17.50 ± 0.05	1.71 ± 0.05	1.18 ± 0.01	0.72 ± 0.05	88.27 ± 0.03
Average	79.45 ± 0.70	17.18 ± 0.13	1.57 ± 0.03	1.27 ± 0.04	0.54 ± 0.04	84.96 ± 0.05
El-Rayah El-Nasery						
Spring	80.26 ± 0.22	17.05 ± 0.05	1.21 ± 0.05	1.18 ± 0.05	0.30 ± 0.05	80.29 ± 0.30
Summer	79.75 ± 0.56	16.95 ± 0.05	1.90 ± 0.02	1.30 ± 0.01	0.10 ± 0.01	85.30 ± 0.10
Autumn	78.66 ± 0.22	17.88 ± 0.03	1.88 ± 0.03	1.39 ± 0.05	0.19 ± 0.01	89.20 ± 0.06
Winter	78.80 ± 0.15	17.69 ± 0.15	1.95 ± 0.03	1.36 ± 0.01	0.20 ± 0.03	89.11 ± 0.03
Average	79.37 ± 0.29	17.39 ± 0.07	1.74 ± 0.03	1.35 ± 0.03	0.20 ± 0.03	85.98 ± 0.12

Table 2 Seasonal variations of physicochemical aspects (on wet weight basis) in muscle tissue of *Oreochromis niloticus* collected from the Nile rayahs.

	pH value	TVB-N (mg/100 g)	TMA (mg/100 g)	TBA (mg MDA/kg)	TBC (Log cfu/g)
El-Rayah El-Tawfiki					
Spring	6.55 ± 0.05	17.73 ± 0.03	0.66 ± 0.01	0.47 ± 0.08	3.20 ± 0.10
Summer	6.70 ± 0.13	19.24 ± 0.05	0.78 ± 0.03	1.18 ± 0.04	3.55 ± 0.05
Autumn	7.21 ± 0.01	17.18 ± 0.04	0.45 ± 0.01	0.68 ± 0.03	3.32 ± 0.04
Winter	6.05 ± 0.01	16.42 ± 0.05	0.38 ± 0.01	0.11 ± 0.03	3.17 ± 0.05
Average	6.63 ± 0.05	17.64 ± 0.04	0.57 ± 0.02	0.61 ± 0.05	3.31 ± 0.06
El-Rayah El-Menoufy					
Spring	6.62 ± 0.03	17.25 ± 0.03	0.80 ± 0.02	1.01 ± 0.03	2.89 ± 0.08
Summer	6.80 ± 0.01	18.38 ± 0.03	0.85 ± 0.03	0.45 ± 0.02	3.82 ± 0.03
Autumn	7.01 ± 0.01	16.08 ± 0.50	0.78 ± 0.02	0.85 ± 0.05	3.65 ± 0.05
Winter	6.95 ± 0.05	16.50 ± 0.67	0.70 ± 0.01	0.70 ± 0.10	2.30 ± 0.03
Average	6.85 ± 0.03	17.05 ± 0.31	0.78 ± 0.02	0.75 ± 0.05	3.17 ± 0.05
El-Rayah El-Behery					
Spring	6.40 ± 0.05	17.84 ± 0.05	0.72 ± 0.03	0.22 ± 0.03	2.47 ± 0.03
Summer	6.64 ± 0.01	17.93 ± 0.03	1.23 ± 0.03	0.58 ± 0.04	3.91 ± 0.05
Autumn	7.19 ± 0.05	16.48 ± 0.03	0.86 ± 0.02	0.90 ± 0.04	2.25 ± 0.05
Winter	7.11 ± 0.04	16.04 ± 0.02	0.77 ± 0.04	0.87 ± 0.02	2.79 ± 0.08
Average	6.84 ± 0.04	17.07 ± 0.03	0.90 ± 0.03	0.64 ± 0.03	2.86 ± 0.05
El-Rayah El-Nasery					
Spring	6.51 ± 0.05	18.45 ± 0.01	0.75 ± 0.04	0.57 ± 0.03	2.25 ± 0.05
Summer	6.77 ± 0.05	18.01 ± 0.03	1.09 ± 0.11	1.11 ± 0.01	3.45 ± 0.05
Autumn	6.90 ± 0.01	17.92 ± 0.03	0.89 ± 0.03	0.94 ± 0.04	3.79 ± 0.06
Winter	6.81 ± 0.05	16.88 ± 0.03	0.67 ± 0.02	0.83 ± 0.03	2.07 ± 0.10
Average	6.75 ± 0.04	17.82 ± 0.03	0.85 ± 0.05	0.86 ± 0.03	2.89 ± 0.07
Permissible level*	NA	30	5	5	10^6

* Egyptian Standard Specifications (2006).

The obtained results indicated that, TVB-N, TMA, TBA and TVC showed a significant decrease during autumn and winter, while they showed a significant increase during spring and summer. Conversely, the pH values increased significantly during autumn and winter in all rayahs. Physicochemical aspects of *O. niloticus* muscles showed a highly spatial and temporal significant difference ($p < 0.01$). Their pH values ranged from (6.05–7.21), TVB-N (16.04–19.24 mg/100 g), TMA (0.38–1.23 mg/100 g), TBA (0.11–1.18 mg MDA/kg) and TBC (2.07–3.91 cfu/g).

In the live fish, the muscle tissue exhibited a pH value close to neutrality, while this value was found to decrease on the first day of death due to the lactic acid anaerobic formation. It would later show a slight increase or consistency because of the basic compounds formation (Love, 1980).

Total volatile basic nitrogen was used as a quality indicator to evaluate the shelf life of fish and fishery products. It is known as a product of bacterial spoilage and endogenous enzymes' action of protein and amino acids (Özogul et al., 2004). The significant increase in TVB-N values during spring and summer and the decrease during autumn and winter may be due to the non-protein nitrogen content, which is related to catching season, surrounding environment, feeding, age, fish size, in addition to the microbiological quality of fish tissue (Durmus et al., 2014). The increase in TVB-N may be attributed to the activity of bacteria and enzymes, hygienic practices and storage conditions (Fernández-Segovia et al., 2007).

Thiobarbituric acid value is widely used as an indicator of the degree of lipid oxidation which affected by processing methods, temperature and storage conditions (Aubourg, 1999). In all samples the TBA values are under the Egyptian permissible levels (Egyptian Standard Specifications, 2006).

Total bacterial counts are the total numbers of bacteria that are capable of forming visible colonies on a culture media at a given temperature, and they are used for evaluating the hygienic quality and the possible presence of pathogenic microorganisms in fish and fish products (Durmus et al., 2014). The changes of TBC during different seasons may be directly related to environmental factors, harvesting, handling, storage and transportation (Ward and Baj, 1988).

Heavy metals concentrations

Seasonal variations of heavy metal concentrations (mg/kg wet weight) in muscle tissue of *O. niloticus* caught from the Nile rayahs during four seasons are shown in (table 3). Generally, the concentrations of the analyzed heavy metals showed irregular distributions with ascending order of: Cd < Pb < Cu < Mn < Zn < Fe.

El-Rayah El-Tawfiki

Fe, Mn and Zn showed higher levels in El-Rayah El-Tawfiki during spring with records of 33.89, 11.16 and 15.75 mg/kg wet weight, respectively. On the other side, Cadmium was not detected in El-Rayah El-Tawfiki, while high levels of Cu

Table 3 Seasonal variations of heavy metal concentrations (mg/kg wet weight basis) in muscle tissue of *Oreochromis niloticus* collected from the Nile rayahs.

	Fe	Mn	Zn	Cu	Pb	Cd
El-Rayah El-Tawfiki						
Spring	33.89 ± 2.67	11.16 ± 0.76	15.75 ± 1.17	nd	0.92 ± 0.018	nd
Summer	14.20 ± 1.48	1.63 ± 0.022	9.84 ± 0.99	1.53 ± 0.013	2.71 ± 0.082	nd
Autumn	10.50 ± 1.11	0.23 ± 0.007	4.29 ± 0.17	1.70 ± 0.019	0.29 ± 0.03	nd
Winter	14.20 ± 2.14	1.27 ± 0.013	1.78 ± 0.021	0.28 ± 0.008	2.55 ± 0.06	nd
Average	18.20 ± 1.85	3.57 ± 0.20	7.92 ± 0.59	0.877 ± 0.01	1.62 ± 0.05	nd
Range in water μg/l*	184.4–723.2	11.89–40.0	19.06–49.83	1.4–4.18	13.4–61.8	0.0–1.56
El-Rayah El-Menoufy						
Spring	13.63 ± 1.16	2.04 ± 0.087	6.72 ± 0.37	0.19 ± 0.003	2.96 ± 0.038	nd
Summer	12.92 ± 1.03	0.74 ± 0.025	6.31 ± 0.49	1.68 ± 0.016	2.72 ± 0.061	nd
Autumn	10.05 ± 0.89	0.81 ± 0.022	1.90 ± 0.053	1.21 ± 0.007	nd	0.41 ± 0.008
Winter	10.88 ± 0.95	2.08 ± 0.037	7.73 ± 0.61	3.95 ± 0.052	nd	nd
Average	11.87	1.42	5.67	1.76	1.42	0.1
Range in water μg/l*	137.4–784.2	16.03–41.07	19.4–55.2	0.8–4.13	12.4–59.4	0.43–2.19
El-Rayah El-Behery						
Spring	15.35 ± 0.98	0.36 ± 0.016	7.73 ± 0.44	nd	1.74 ± 0.048	nd
Summer	177.19 ± 9.4	8.97 ± 0.48	19.52 ± 0.93	1.19 ± 0.029	0.32 ± 0.009	nd
Autumn	19.15 ± 1.06	2.83 ± 0.17	2.15 ± 0.09	1.31 ± 0.017	2.38 ± 0.043	0.32 ± 0.008
Winter	43.76 ± 2.06	1.90 ± 0.026	8.10 ± 0.52	0.70 ± 0.008	1.94 ± 0.016	nd
Average	63.86	3.52	9.38	0.800	1.595	0.08
Range in water μg/l*	157.8–915.5	13.86–65.4	19.27–74.2	0.91–7.18	12.0–59.6	0.0–1.47
El-Rayah El-Nasery						
Spring	13.98 ± 0.87	0.87 ± 0.067	11.53 ± 0.98	1.20 ± 0.008	1.32 ± 0.009	nd
Summer	8.24 ± 0.42	1.11 ± 0.021	8.89 ± 0.75	1.36 ± 0.008	0.18 ± 0.002	nd
Autumn	20.32 ± 1.68	0.94 ± 0.018	4.64 ± 0.23	1.09 ± 0.006	nd	0.30 ± 0.005
Winter	14.16 ± 1.03	1.14 ± 0.063	7.83 ± 0.76	3.88 ± 0.017	1.10 ± 0.011	nd
Average	14.18	1.02	8.22	1.883	0.650	0.08
Range in water μg/l*	152.8–760.6	12.4–47.4	17.89–72.6	1.4–4.2	11.2–46.0	0.26–2.07
Permissible level**	100	5	40	30	2	0.5

*Goher (2015), NA: not available, ** (WHO, 1993, mg/kg wet weight).

Table 4 Comparison between heavy metal content in *Oreochromis niloticus* muscles in the present work and earlier studies.

Reference	Fe	Mn	Zn	Cu	Pb	Cd
Ibrahim et al. (2008)*	nd	nd	2.9–4.5	1.13–2.15	0.6–3.5	0.4–1.2
Saeed (2013)**	39–89.4	2.2–2.8	23.74–47.2	2.0–3.1	0.14–0.92	0.13–0.49
Younis et al. (2014)**	nd	nd	0.29–0.41	nd	1.63–1.96	3.14–4.14
Present results*	19.2–20.8	1.2–3.61	3.25–11.1	0.70–2.2	1.341.86	Nd–0.34

* mg/kg wet weight.
** mg/kg dry weight.

(1.70 mg/kg wet weight) and Pb (2.71 mg/kg wet weight) were recorded during autumn and summer, respectively.

El-Rayah El-Menoufy

El-Rayah El-Menoufy showed the highest values of Fe and Pb (13.63 and 2.96 mg/kg wet weight) during spring, while Mn, Zn, Cu exhibited a significant increase during winter with records of 2.08, 7.73 and 3.95 mg/kg wet weight, respectively.

El-Rayah El-Behery

Higher concentrations of Fe, Mn and Zn (177.19, 8.97 and 19.52) mg/kg wet weight, respectively, were recorded in El-Rayah El-Behery during summer, Moreover, El-Rayah El-Behery showed higher levels of Cu (1.31), Pb (2.38) and Cd (0.32) mg/kg wet weight, during autumn.

El-Rayah El-Nasery

El-Rayah El-Nasery showed high concentrations of Fe 20.32 and Cd and 0.30 mg/kg wet weight during autumn, Mn and Cu recorded their highest values as 1.14 and 3.88 mg/kg wet weight during winter. Finally, Zn and Pb were higher during spring as they recorded 11.53 and 1.32 mg/kg wet weight, respectively.

According to the regional average levels of the studied metals, muscles of fishes caught during winter and summer seasons

Table 5 A correlation matrix between proximate chemical composition, physicochemical, microbiological and heavy metals in *Oreochromis niloticus*.

	Moisture	Protein	Fat	Ash	Carbohydrates	Calorific value	pH	TVBN	TMA	TBA	TVC	Fe	Mn	Zn	Cu	Pb	Cd
Moisture	1																
Protein	−0.780	1															
Fat	−0.715	0.430	1														
Ash	−0.234	−0.160	0.122	1													
Carbohydrates	−0.364	−0.013	0.000	0.012	1												
Calorific value	−0.942	0.760	0.860	0.005	0.264	1											
pH	−0.366	0.312	0.180	0.121	0.147	0.308	1										
TVBN	0.627	−0.413	−0.115	−0.332	−0.578	−0.431	−0.307	1									
TMA	0.244	−0.618	0.109	0.433	−0.008	−0.230	0.175	0.342	1								
TBA	−0.104	0.005	0.343	−0.168	0.035	0.236	0.589	0.089	0.422	1							
TVC	0.288	−0.418	−0.057	0.048	−0.009	−0.241	−0.012	0.342	0.337	0.092	1						
Fe	0.164	−0.582	0.042	0.379	0.257	−0.185	−0.079	0.098	0.575	−0.125	0.334	1					
Mn	0.278	−0.497	−0.078	0.225	0.046	−0.279	−0.167	0.097	0.290	−0.160	0.196	0.653	1				
Zn	0.518	−0.650	−0.128	0.068	−0.156	−0.440	−0.251	0.493	0.486	−0.060	0.125	0.694	0.744	1			
Cu	−0.424	0.296	0.379	0.105	0.132	0.426	0.416	−0.133	−0.006	0.266	−0.313	−0.112	−0.238	−0.062	1		
Pb	0.248	−0.123	−0.079	−0.403	−0.053	−0.125	−0.324	0.132	−0.216	−0.111	−0.172	−0.200	−0.149	−0.183	−0.344	1	
Cd	−0.406	0.264	0.037	0.337	0.325	0.240	0.432	−0.342	0.151	0.283	0.156	−0.133	−0.143	−0.510	−0.052	−0.256	1

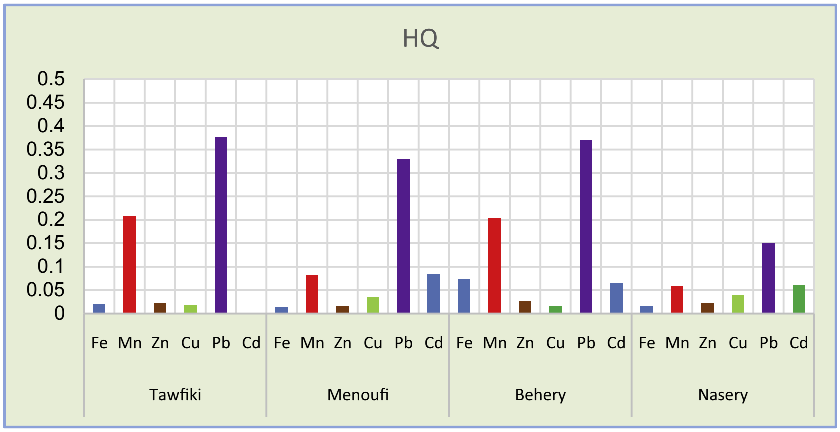

Figure 2 Hazard Quotient (HQ) to humans due to individual metal through the muscles of *Oreochromis niloticus* from The Nile rayahs.

had more heavy metals than those in autumn and spring. The obtained results disagreed with the previous results obtained by Ibrahim et al. (2008), Saeed (2013) and Younis et al. (2014) (Table 4), they recorded the highest levels of metals in the muscles of Nile tilapia during the cold seasons (autumn and winter).

The low concentration of Cd might be due to its low tendency for bioaccumulation or its good ability of excretion from the body. These findings are similar to those obtained by Ibrahim et al. (2008).

It is obvious that, the levels of all the detected heavy metals were under the permissible values recommended by WHO (1993), so a normal daily diet including the studied fish species poses no health risk to consumers.

An ANOVA did not show either a temporal or a spatial significant difference for the levels of the analyzed metals in the muscles of *O. niloticus* in the four rayahs, except for Zn that showed a temporal significant difference ($p < 0.05$). Overall, this observation may be related to the fact that there aren't any significant differences between these metals in the rayahs' water and to also the fact that they possess low concentrations (Goher, 2015). Generally, the concentrations of heavy metals in fish depend on feeding habits (Canli and Atli, 2003; Uysal et al., 2009), size, age (Rashed, 2001; Fernandes et al., 2007), lifestyle and exposure durations to contaminants of different species. (Canli and Kalay, 1998).

On the other side, the correlation coefficient (Table 5) recorded a positive correlation between iron and manganese ($r = 0.653$, $p < 0.05$), between iron and zinc ($r = 0.694$, $p < 0.05$) and manganese and zinc ($r = 0.744$, $p < 0.01$). These results may reflect a common source of occurrence and indicate similar biogeochemical pathways for subsequent accumulation in the muscle tissue of fishes (Rejomon et al., 2010). In contrast, zinc is negatively correlated with cadmium ($r = -0.51$, $p < 0.05$). The concentrations of the toxic elements in fish are mainly affected by the levels of these metals in the habitats, the food of the fish and the rate of metal detoxification (Urena et al., 2007).

Hazard Indices and Human Health Risk

Human Health Risk Assessment (HHRA) addresses potential impacts to people exposed to toxic elements. It is Incumbent to evaluate public health risks emerging from the existence of toxic chemicals in food. To assess this Hazard Index, a comparison of dietary intake of contaminants for corresponding reference intakes, must be made . Thus, an assessment of the actual dietary intake of toxic elements is very useful for risk assessment. (WHO, 1999).

The present study utilized the US EPA pattern and their acceptable limits to evaluate the possible human health risks that may be caused by heavy metal uptake that is associated

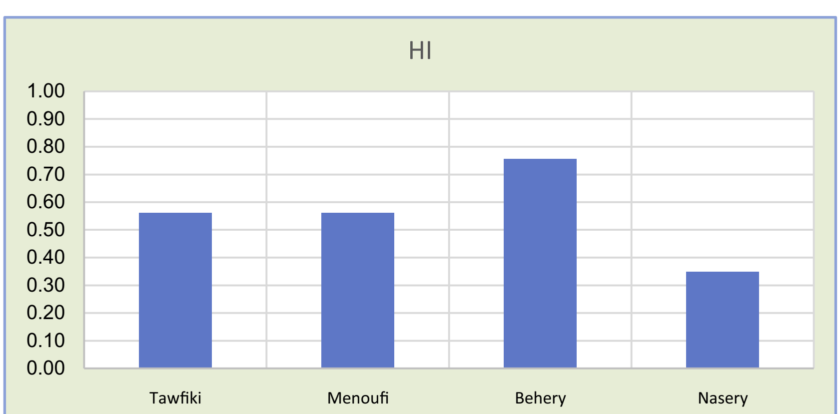

Figure 3 Hazard Index (HI) to human population from metals through muscles of *Oreochromis niloticus* from The Nile rayahs.

with the consumption of *O. niloticus* caught from the Nile rayahs, Egypt. The risk of human health models containing non-carcinogenic and carcinogenic substances developed by US EPA has demonstrated efficacy and has been accepted worldwide (Osakwe et al., 2014).

Hazard Index (HI) and Hazard Quotient (HQ) are used to evaluate the health risk for humans from fish intake (Goher et al., 2015). The following equations are used to compute the HI and HQ values according to United States Environmental Protection Agency (USEPA, 2013).

$$HI = \sum_{i=1}^{n} HQ_i \quad (1)$$

where HQ_i is the Hazard Quotient for the individual heavy metal.

$$HQ = 10^{-3} \times \frac{C \times EF \times ED \times FI}{RFD_o \times BW \times AT} \quad (2)$$

where, C: the concentration of metal in fish tissue (milligrams per kilogram, ww); EF: exposure frequency; FI: intake rate of fish per person per day (57 g in Egypt, FAO, 2014) and ED: the life time exposure duration (70 years); BW is the body weight (70 kg is used as a default value for the adult, USEPA, 2013); AT is averaging time for noncarcinogens (365 days/year × number of exposure years); RFD_o is the oral reference dose (ppm per day); and 10^{-3} is the unit conversion factor.

The HQ is a ratio of estimated dosage of a pollutant to a reference dosage level (Goher et al., 2015). HQ is known as the measurable magnitude of the possibility of health effects of non-carcinogenic for an average exposure period; it is derived by summing the Hazard Quotient values of the elements. This total HQ is expressed as the (HI) Hazard Index (Kumar et al., 2013). When HQ is equal to or less than one ($\leqslant 1$), it indicates no appreciable health risk, while if $HQ > 1$, then it indicates a reason for health concern (USEPA, 2013). On the other hand, no health risk may occur as a result of ingestion of the fish at Hazard Quotient (HQ) or total Hazard Index (HI) below one. The greater the value of (HQ) and (HI) (if it is above 1), the greater the level of risk associated with fish consumption will be. Hence, $HI < 1$ means no hazard; $1 > HI < 10$ means moderate hazard while greater than 10 means high hazard or risk (Ukoha et al., 2014).

The calculated HQ and HI values are given in Figs. 2 and 3. The HQ due to ingestion of the studied metals through *O. niloticu* from the Nile rayahs was in the range of Fe (HQ = 0.014–0.074), Mn (HQ = 0.059–0.208), Zn (HQ = 0.015–0.025), Cu (HQ = 0.014–0.074), Pb (HQ = 0.014–0.074) and Cd (HQ = 0.014–0.074). That indicates – based on the Average HQ value – that the lead has the highest HQ value, while zinc recorded the lowest one.

HI values due to consumption of *O. niloticu* from the four rayahs were in following sequence, HI = 0.76 for El Rayah El Behery, 0.64 for El Rayah El Tawfiki, 0.65 for El Rayah El Menoufy and 0.35 for El Rayah El Nasery.

The HQ values calculated for consumption were $HQ < 1$, in fact several times lower than the risk index of 1. The Hazard Indexes of total non-carcinogenic (HI) for the analyzed heavy metals were lower than the acceptable limit (HI = 1), which was classified as no hazard ($HQ < 1$) (Ukoha et al., 2014).

In general, the levels of metals found in *O. niloticu* caught from the Nile rayahs do not pose any particular health risk concern due to low values of HQ and HI obtained for all the analyzed metals.

Conclusion

Heavy metals, TVB-N, TMA, TBA and TVC didn't exceed the maximum permissible limits of (WHO, 1993 and Egyptian Standard Specifications, 2006) in the tissues of *O. niloticu* collected from four rayahs. Also, the values of Hazard Index (HI) and Hazard Quotient (HQ) were found to be lower than the acceptable limits. These results indicate that the metals in *O. niloticu* in the Nile rayahs, Egypt, do not pose any particular human health risk concern. So, *O. niloticus* muscles collected from the four rayahs are safe for human consumption.

Conflict of interest

There is no conflict of interest.

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