



Seasonal variation of heavy metals in water and organs of *Oreochromis niloticus* at Rosetta Branch, River Nile, Egypt.

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ABSTRACT

The pollution of the aquatic environment with heavy metals is one of the most principal water, aquatic life, and public health problems in the Rosetta Branch. There are annual increases in pollutants and heavy metals that flow into the River Nile by many sources; as agricultural and industrial activities as well as sewage wastewater. The present study aimed to evaluate the seasonal variation of heavy metals contents (Pb, Cd, Hg, Cr, Fe, and Zn) in water and Nile tilapia fish samples, which collected from Rosetta Branch, River Nile and provide information regarding fish growth and water quality. This study was carried out along the Rosetta Branch at four sites (Kafr El-Zayat, Minyat Jenaj, Desouk, and Motobas) during 2016/2017 in four seasons. The results revealed that winter was the highest season in heavy metal concentration and summer was the lowest in heavy metals concentrations. Additionally, Kafr El-Zayat was the highest site in heavy metals concentration, and Minyat Jenaj was the lowest. In water samples, Hg was higher than the permissible limit in all season and sites. Moreover, Pb concentration was higher than the allowable limit in all seasons and sites except summer; Cd concentration was higher than the acceptable limit in autumn and winter seasons. Concerning fish samples, heavy metals concentrations in gills were much higher than that of the muscles. In Desouk and Motobas sites, the concentration of Pb in muscles was higher than the permissible limit in autumn and winter seasons. The concentration of Cd in muscles was higher than the permissible limit in autumn at Kafr El-zayat; spring season gave the highest values of HSI comparing to other seasons.

INTRODUCTION

The pollution of aquatic ecosystems by heavy metals is a significant environmental problem, as heavy metals constitute some of the most hazardous substances that can bio-accumulate in various biotic systems, (Van den Broek *et al.*, 2002). Heavy metals are ubiquitous in the environment because of both natural and

anthropogenic activity, and humans are exposed to them through various pathways, especially food chain, (Harmanescu *et al.*, 2011).

There are two primary sources of water pollution of Rosetta Branch, agricultural drainage water from five major drains; El-Rahawy, Sabal, El-Tahrir, Zaweit El-Bahr, and Tala, directly discharge in this branch. These drains fetch animal and domestic wastes, sediments, inorganic salts, crop residues, minerals, chemical fertilizers, and pesticides (Donia, 2005). The second source of pollution is the industrial wastewater outfalls produced by mega-companies in Kafr El-Zayat city; these industrial outfalls are Salt and Soda, El-Mobidat and El-Malyia companies which are discharged directly at the east bank of the branch without any treatment, (Usali and Ismail, 2010).

Assessment and impact relationship of the effects of heavy metals in biological species are essential for the preservation of the aquatic ecosystem. Therefore, heavy metals pose severe threats due to their potential to enter aquatic organisms, and for their bio-accumulation and bio-magnification in the food chain (Dhanakumar *et al.*, 2015). Fish is highly recommended as an animal protein source to avoid high cholesterol level, consequently the quality of fish is of particular concern. Moreover, fish is successfully employed in bio-monitoring programs of a wide range of pollutants, including heavy metals in order to assess the quality of the marine environment (Kuklina *et al.*, 2014). Knowledge of heavy metals concentration in fish is vital concerning nature the management and human consumption fish. Heavy metals enter the aquatic food chain through the digestive tract and non-dietary routes across permeable membranes such as the muscles and gills directly consumption of waters and food, (Rajeshkumar and Li, 2018). The higher concentration of heavy metals beyond the tolerance limit of fishes affects fish populations, reducing their growth, reproduction and/or survival and may even kill fishes, (Elghobashy *et al.*, 2001).

Fish accumulate pollutants directly from diet and water, and contaminant residues may very well reach concentrations thousands of times above those measured in the food and water, (Osman *et al.*, 2007). Also, it preferentially in their fatty tissues like liver, and the effects become apparent when concentrations in such tissues attain a threshold level, (Omar *et al.*, 2014). For this reason, monitoring fish tissue contamination serves as an important function and early warning indicator of water quality problems (Mansour and Sidky, 2002). Monitoring of water quality using fish as bio-indicators enables us to take appropriate action to protect public health and environment. Also, condition factor (CF) and hepato-somatic index (HSI) are used as a biomarker of fish health in relation to pollution in general and heavy metals in particular. This is strongly influenced by both biotic and abiotic environmental conditions such as feeding intensity, growth rates, and fish age, (Javed and Nazura, 2019).

The present study aimed to evaluate the seasonal variation of heavy metals contents (Pb, Cd, Hg, Cr, Fe, and Zn) in Nile tilapia (*Oreochromis niloticus*) fish and water samples which collected from Rosetta Branch and provide information regarding the growth of fish and water quality.

MATERIALS AND METHODS

The study area

This study was conducted at Rosetta Branch, Nile River, which located at the western part of the Nile Delta. Its length is about 220.0 km, average width 180.0 m

with an average depth varies between 1.5 - 16.0 m. It serves five governorates of the Nile Delta, Qalyoubia, El-Menofyia, El-Gharbia, Kafr El-Sheikh, and El-Behira, (El-Amier *et al.*, 2015). The study area was divided throughout the Rosetta Branch into four sites (Kafr El-Zayat, Minyat Jenaj, Desouk, and Motobas), as shown in Fig. (1).

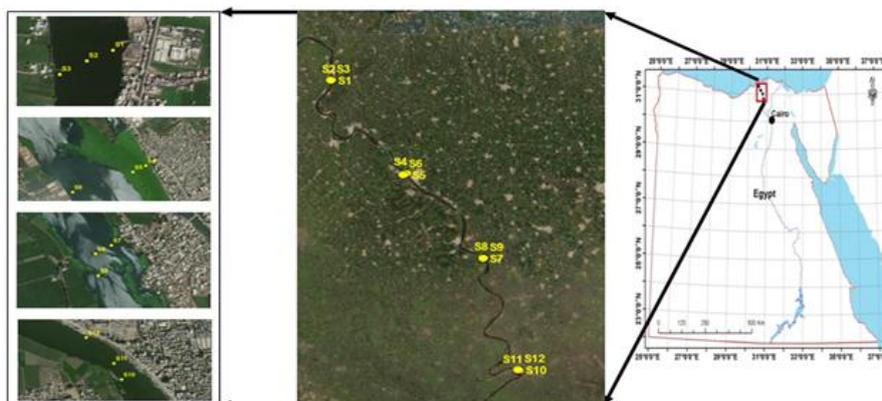


Fig. 1: The study area and sampling location at Rosetta Branch

Water analysis

Water samples were collected seasonally from August 2016 till May 2017 from four sites at the Rosetta Branch (Kafr El-Zayat, Minyat Jenaj, Desouk, and Motobas) (Fig. 1). For each site, three water samples were collected from the center and both side at a depth of 30.0 cm, and then they were kept acidified by concentrated nitric acid (HNO_3) and stored at 4°C until analysis. Heavy metals concentrations (Pb, Cd, Cr, Fe, and Zn) determined after filtration by atomic absorption spectrophotometer (Agilent technologies 200 series AA and GTA 120 Graphite tube atomizer), also the Hg determined by using NIC MR- 3000 mercury analyzer, according to APHA (2012).

Fish analysis

Nile tilapia fish (*Oreochromis niloticus*) samples were collected seasonally from August 2016 till May 2017, from the four sites at Rosetta Branch (Kafr El-Zayat, Minyat Jenaj, Desouk, and Motobas) which captured by fishermen. Fish gills and muscles tissues have been digested using microwave digestion methods using the (High-performance microwave digestion system- ETHOS one) according to EPA (2007). Then the heavy metals concentration in gills and muscles were measured as the same methods used for water analysis.

Fish length and weight, also condition factor was determined according to the formula stated by Salam and Davies (1994).

$$\text{CF} = (\text{W}/\text{L}^3) \times 100$$

Where:

CF= condition factor, W = total weight (g) and L=total length (cm)

When CF value is higher, it means that the fish has attained a better condition, (Nehemia *et al.*, 2012)

Hepato-somatic index (HSI) was calculated by liver weight and body weight ratio using the following formulae, according to Wingfield and Grimm (1977).

$$\text{HSI} = \text{W}_{\text{liver}} / \text{W}_{\text{guttated}} \times 100.$$

Where:

$\text{W}_{\text{guttated}}$ = the fish gutted weight, gutted weight (takeout the internal organs).

The hepato-somatic index is that the health condition is essential for the growth of fish. HSI might be useful as an indicator of chemical water pollution; higher HSI value means fishes are overgrowing and have an excellent aquatic environment, and

if HSI value is less, it means fish is not growing well, and it is facing unhealthy environmental problems, (Pyle *et al.*, 2005).

Statistical analysis

Two-way analysis of variance (ANOVA) was used to indicate significant difference in season and site, ANOVA was followed by Duncan's multiple range test (Duncan, 1955) at significantly of (5%). All statistical calculations were carried out with using SPSS 20" for windows.

RESULTS AND DISCUSSION

Monitoring of heavy metals in water at Rosetta branch

The results of heavy metals concentration in water samples in the selected sites are given in Table (1). The values of Pb were ranged between 0.0001 to 0.096 mg L⁻¹. They were higher in winter in Motobas and lower in summer in Kafr El-Zayat. The record values were higher than the permissible limit except in the summer season.

Table 1: Concentration of heavy metals in water samples (mg L⁻¹) at the Rosetta Branch during 2016/ 2017

Season	Site	The concentration of heavy metals (mg L ⁻¹)					
		Pb	Hg	Cd	Zn	Fe	Cr
Summer 2016	Kafr El-Zayat	0.0001 ^d ±	0.0046 ^b ±	0.00003 ^d ±	0.000070 ^d ±	0.0276 ^c ±	0.0016 ^c ±
		0.0001	0.00037	0.00001	0.00005	0.0026	0.0004
	Minyat Jenaj	0.0020 ^d ±	0.0067 ^b ±	0.00003 ^d ±	0.000333 ^d ±	0.0278 ^c ±	0.0018 ^c ±
		0.0010	0.00301	0.00001	0.00004	0.0060	0.0004
	Desouk	0.0007 ^d ±	0.0054 ^b ±	0.00002 ^d ±	0.000289 ^d ±	0.0242 ^c ±	0.0018 ^c ±
0.0004		0.00095	0.00000	0.00008	0.0086	0.0008	
Motobas	0.0004 ^d ±	0.0055 ^b ±	0.00002 ^d ±	0.000277 ^d ±	0.0125 ^c ±	0.0016 ^c ±	
	0.0006	0.00061	0.00001	0.00022	0.0075	0.0006	
	Mean	0.0009^C ±	0.0055^B ±	0.000024^C ±	0.000242^C ±	0.0230^C ±	0.0017^D ±
		0.0009	0.00159	0.000007	0.00014	0.0086	0.0005
Autumn 2016	Kafr El-Zayat	0.0273 ^{bcd} ±	0.0146 ^{ab} ±	0.0019 ^c ±	0.012600 ^{cd} ±	0.2958 ^a ±	0.0630 ^f ±
		0.0015	0.00366	0.0002	0.00372	0.2273	0.0010
	Minyat Jenaj	0.0296 ^{bcd} ±	0.0385 ^a ±	0.0024 ^c ±	0.010033 ^{cd} ±	0.0895 ^c ±	0.0516 ^d ±
		0.0015	0.04306	0.0005	0.00062	0.0169	0.0011
	Desouk	0.0260 ^{bcd} ±	0.0173 ^{ab} ±	0.0025 ^{bc} ±	0.009983 ^{cd} ±	0.0895 ^c ±	0.0503 ^d ±
0.0017		0.00939	0.0002	0.00200	0.0030	0.0032	
Motobas	0.0276 ^{bcd} ±	0.0240 ^{ab} ±	0.0028 ^{bc} ±	0.008417 ^{cd} ±	0.0813 ^c ±	0.0460 ^d ±	
	0.0015	0.01666	0.0002	0.00197	0.0121	0.0020	
	Mean	0.0276^B ±	0.0236^A ±	0.0024^B ±	0.010258^B ±	0.1408^B ±	0.0527^B ±
		0.00192	0.02235	0.0004	0.00254	0.1350	0.0067
Winter 2017	Kafr El-Zayat	0.063 ^{abc} ±	0.0258 ^{ab} ±	0.0030 ^{bc} ±	0.027000 ^{ab} ±	0.2556 ^{ab} ±	0.1000 ^a ±
		0.0333	0.00514	0.0026	0.01915	0.0183	0.0173
	Minyat Jenaj	0.091 ^a ±	0.0192 ^{ab} ±	0.0043 ^a ±	0.004333 ^{cd} ±	0.1456 ^{bc} ±	0.0870 ^b ±
		0.06209	0.00616	0.0005	0.00115	0.0326	0.0185
	Desouk	0.071 ^{ab} ±	0.0169 ^{ab} ±	0.0036 ^{ab} ±	0.007000 ^{cd} ±	0.3140 ^a ±	0.0813 ^b ±
0.0102		0.00252	0.0005	0.00173	0.1257	0.0020	
Motobas	0.096 ^a ±	0.0174 ^{ab} ±	0.0046 ^a ±	0.013000 ^{cd} ±	0.3076 ^a ±	0.0653 ^c ±	
	0.0623	0.00210	0.0005	0.00360	0.1393	0.0083	
	Mean	0.080^A ±	0.0198^A ±	0.0039^A ±	0.012833^B ±	0.2557^A ±	0.0834^A ±
		0.0427	0.00526	0.0013	0.01239	0.1078	0.0173
Spring 2017	Kafr El-Zayat	0.0200 ^{cd} ±	0.0253 ^{ab} ±	0.0001 ^d ±	0.035667 ^a ±	0.0133 ^c ±	0.0033 ^c ±
		0.0057	0.00185	0.0001	0.00585	0.0041	0.0173
	Minyat Jenaj	0.0233 ^{cd} ±	0.0179 ^{ab} ±	0.0002 ^d ±	0.036000 ^a ±	0.0103 ^c ±	0.0093 ^c ±
		0.0019	0.01317	0.0001	0.00793	0.0119	0.0032
	Desouk	0.0261 ^{bcd} ±	0.0235 ^{ab} ±	0.0001 ^d ±	0.027333 ^{ab} ±	0.0176 ^c ±	0.0073 ^c ±
0.0047		0.00342	0.0001	0.01159	0.0050	0.0037	
Motobas	0.0280 ^{bcd} ±	0.0147 ^{ab} ±	0.0002 ^d ±	0.015667 ^{bc} ±	0.0270 ^c ±	0.0103 ^c ±	
	0.0060	0.00256	0.0001	0.00665	0.0050	0.0040	
	Mean	0.0243^B ±	0.0203^A ±	0.0001^C ±	0.028667^A ±	0.0170^C ±	0.0075^C ±
		0.0053	0.00742	0.0001	0.01117	0.0090	0.0040
All over mean	Kafr El-Zayat	0.0277 ±	0.0176 ±	0.0012 ±	0.018834 ^a ±	0.1481 ^a ±	0.0419 ^a ±
		0.0279	0.00954	0.0017	0.01666	0.1657	0.0040
	Minyat Jenaj	0.0365 ±	0.02060 ±	0.0017 ±	0.012675 ^b ±	0.0700 ^b ±	0.0374 ^{ab} ±
		0.0435	0.0228	0.0018	0.01491	0.0590	0.0374
	Desouk	0.0310 ±	0.01578 ±	0.0015 ±	0.011151 ^b ±	0.1113 ^{ab} ±	0.0352 ^{bc} ±
0.0270		0.00813	0.0016	0.01159	0.1367	0.0341	
Motobas	0.0380 ±	0.01545 ±	0.0019 ±	0.009340 ^b ±	0.1071 ^{ab} ±	0.0308 ^c ±	
	0.0455	0.01003	0.0020	0.00695	0.1375	0.02740	
	permissible limits^a	0.01	0.001	0.001	0.01	0.5	0.05

* Egyptian Ministry of Water Resources and Irrigation law 48/1982, for protection of the Nile River and waterways from pollution (i.e., decree No. 49 in the amended executive regulations of the law by Minister Decision No. 92/2013).

- Means values (mean ± SD, n = 3) within a column with a same superscript are not significantly different (P > 0.05).

The results of Hg concentration showed the highest value in all seasons and sites under investigation (0.00046 to 0.0385 mg L⁻¹). The concentration of Cd showed the highest value in winter in Motobas (0.0046 mg L⁻¹) with the lowest figures in the summer. Regarding to the Zn concentration, the results showed that the highest value (0.036 mg L⁻¹) was recorded in spring season in Minyat Jenaj site while the lowest value (0.00007 mg L⁻¹) was detected in summer season in Kafr El-Zayat site, and the all values were less than the permissible limit except in winter season. Concerning of Fe concentration, the obtained data indicated that the highest value (0.314 mg L⁻¹) was observed in winter season in Desouk site while the lowest value (0.0103 mg L⁻¹) was mentioned in spring season in MinyatJenaj site, and the all values were less than the permissible limit. Finally; the results of Cr revealed that the highest concentration was recorded in winter season in Kafr El-Zayat site, while the lowest value was recorded in summer season in Kafr El-Zayat and Motobas and all these values were lower compared to the permissible limit in all seasons and sites except in winter. These results are in agreement with those obtained by Abdo (2006), who revealed that the values of trace metal increased during cold seasons than hot seasons. This is mainly attributed to the increasing mobilization of trace elements from sediment to water and closure of canals (sadda Elshetweyah) which reduces freshwater availability. Also, El-Sayed *et al.* (2011) found that there is a significant difference in water quality as a result of annual variations in heavy metals concentrations. The maximum concentrations were observed in spring and winter, while the minimum values were detected in summer and autumn in all heavy metals. These results disagree with Ibrahim and Omar (2013) who recorded that summer season was the highest heavy metals concentrations; however the lowest values detected in winter which collected from Assiut Governorate. Seasonal variations may be attributed to the fluctuation of the amount of untreated domestic sewage, industrial wastes discharged and agricultural drainage water into the drains and canals which feed ponds (Authman *et al.*, 2008). High level of heavy metals concentrations may be due to water contamination from superphosphate, Salt and Soda Company and pesticides factories in Kafr EI Zayat city, quarrying (especially limestone), cement-making, and brick-making, as well as, sewage effluents, agricultural discharges and high ways or motorboat traffic from Kafer El-Zayat to Motobas and release of industrial effluents into the water bodies without sufficient treatment.

Heavy metals contents in fish organs at the Rosetta Branch

Heavy metals contents in fish gills

The results of heavy metals concentration in fish gills samples in different seasons and the studied sites are given in Table (2). The values of Pb were ranged between 0.097 - 1.8223 mg kg⁻¹, and the Desouk site was recorded the highest and lowest values during winter and spring season, respectively. Regarding with Hg levels, the obtained data showed that the concentration ranged between 0.060 - 0.481 mg kg⁻¹, and it was showed higher value in spring season in Minyat Jenaj site, while it was lower in summer season in Motobas site. Also, the concentration of Cd showed a wide range of variation between 0.009 - 0.298 mg kg⁻¹, it was higher in autumn season in Minyat Jenaj site, while it was lower in summer season in Kafr El-Zayat site. Concerning the concentration of Zn in fish gills, the obtained results also showed a wide range of variation between 0.015 - 12.5 mg kg⁻¹, whereas the highest value was detected in winter season in Desouk site, while the lowest value was recorded in summer season in Minyat Jenaj site. Regarding with Fe analysis, the obtained results revealed that the concentration ranged between 0.971-33.368 mg kg⁻¹, and the highest

value was observed in spring season in Kafr El-Zayat site, while the lowest value was recorded in autumn season in Kafr El-Zayat site. Finally; the concentration of Cr showed a wide range of variation between 0.047 - 3.719 mg kg⁻¹, and the concentration was higher in summer season in Motobas site, while the lower concentration was observed during autumn season in Kafr El-Zayat site.

Table 2: Concentration of heavy metals in gills fish samples (mg L⁻¹) at the Rosetta Branch during 2016/2017.

Season	Site	The concentration of heavy metals (mg kg ⁻¹)					
		Pb	Hg	Cd	Zn	Fe	Cr
Summer 2016	Kafr El-Zayat	0.209 ^d ± 0.016	0.211 ^{cde} ± 0.023	0.009 ^c ± 0.004	0.017 ^c ± 0.004	2.455 ^{fg} ± 0.126	1.399 ^c ± 0.563
	Minyat Jenaj	0.252 ^d ± 0.123	0.171 ^{def} ± 0.042	0.244 ^{ab} ± 0.408	0.0150 ^e ± 0.004	2.277 ^{fg} ± 0.119	0.8100 ^d ± 0.359
	Desouk	0.210 ^d ± 0.049	0.110 ^{ef} ± 0.041	0.020 ^c ± 0.001	0.099 ^e ± 0.086	2.358 ^{fg} ± 0.155	2.242 ^b ± 0.243
	Motobas	0.192 ^d ± 0.004	0.060 ^f ± 0.002	0.017 ^c ± 0.004	0.019 ^e ± 0.004	2.219 ^{fg} ± 0.187	3.719 ^a ± 0.479
	Mean	0.216^C± 0.061	0.138^C± 0.066	0.073^B± 0.202	0.037^D± 0.052	2.327^C± 0.157	2.042^A± 1.199
Autumn 2016	Kafr El-Zayat	1.203 ^{abc} ± 0.195	0.183 ^{def} ± 0.016	0.204 ^{abc} ± 0.019	0.937 ^c ± 0.052	0.971 ^g ± 0.126	0.047 ^c ± 0.019
	Minyat Jenaj	1.646 ^{ab} ± 0.463	0.157 ^{def} ± 0.048	0.298 ^a ± 0.058	0.955 ^c ± 0.009	1.806 ^g ± 0.481	0.048 ^c ± 0.010
	Desouk	1.386 ^{ab} ± 0.392	0.268 ^{cd} ± 0.143	0.258 ^{ab} ± 0.052	0.787 ^c ± 0.055	1.369 ^g ± 0.129	0.164 ^c ± 0.097
	Motobas	1.556 ^{ab} ± 0.174	0.424 ^{ab} ± 0.027	0.264 ^{ab} ± 0.071	0.888 ^c ± 0.095	1.272 ^g ± 0.069	0.308 ^c ± 0.037
	Mean	1.448^A± 0.333	0.258^B± 0.127	0.256^A± 0.058	0.892^C± 0.086	1.354^C± 0.383	0.142^B± 0.121
Winter 2017	Kafr El-Zayat	1.715 ^{ab} ± 0.145	0.206 ^{cde} ± 0.022	0.080 ^{bc} ± 0.126	9.167 ^b ± 0.599	13.564 ^d ± 1.449	0.052 ^e ± 0.010
	Minyat Jenaj	1.419 ^{ab} ± 0.096	0.321 ^{bc} ± 0.095	0.156 ^{abc} ± 0.038	3.925 ^d ± 0.510	3.286 ^{fg} ± 0.578	0.074 ^e ± 0.008
	Desouk	1.8223 ^a ± 0.464	0.232 ^{cde} ± 0.021	0.020 ^c ± 0.005	12.500 ^a ± 0.488	25.297 ^b ± 1.149	0.065 ^e ± 0.009
	Motobas	1.323 ^{ab} ± 0.218	0.182 ^{def} ± 0.013	0.017 ^c ± 0.015	10.518 ^b ± 0.601	15.959 ^c ± 2.947	0.053 ^e ± 0.006
	Mean	1.571^A± 0.315	0.235^B± 0.069	0.068^B± 0.082	9.0278^A± 3.349	14.527^A± 8.317	0.061^B± 0.012
Spring 2017	Kafr El-Zayat	0.617 ^{cd} ± 0.23	0.271 ^{cd} ± 0.043	0.015 ^c ± 0.005	6.593 ^c ± 3.153	33.368 ^d ± 2.516	0.055 ^e ± 0.054
	Minyat Jenaj	1.192 ^{abc} ± 0.32	0.481 ^a ± 0.172	0.013 ^c ± 0.018	6.895 ^c ± 1.027	4.765 ^{ef} ± 0.919	0.100 ^e ± 0.005
	Desouk	0.097 ^d ± 0.22	0.244 ^{cd} ± 0.044	0.030 ^c ± 0.005	6.957 ^c ± 1.464	5.840 ^e ± 3.205	0.050 ^e ± 0.020
	Motobas	1.115 ^{bc} ± 1.09	0.274 ^{cd} ± 0.023	0.013 ^c ± 0.010	6.442 ^c ± 1.530	2.783 ^{fg} ± 1.179	0.060 ^e ± 0.035
	Mean	0.755^B± 0.677	0.318^A± 0.127	0.018^B± 0.012	6.722^B± 1.692	11.689^B± 13.253	0.0662^B± 0.035
All over mean	Kafr El-Zayat	0.936± 0.615	0.217^b± 0.041	0.077^b± 0.096	4.179^b± 4.223	12.589^a± 13.578	0.388^c± 0.655
	Minyat Jenaj	1.127± 0.608	0.282^a± 0.163	0.178^a± 0.244	2.848^c± 2.859	3.034^d± 1.228	0.258^c± 0.367
	Desouk	0.879± 0.818	0.213^b± 0.092	0.082^b± 0.119	5.086^a± 5.310	8.716^b± 10.252	0.630^b± 0.979
	Motobas	1.047± 0.724	0.235^{ab}± 0.139	0.078^b± 0.129	4.467^{ab}± 4.520	5.559^c± 6.442	1.035^a± 1.635

- Means values (mean ± SD, n = 3) within a column with a same superscript are not significantly different (P > 0.05).

Heavy metals content in fish muscles

Fish is generally appreciated as one of the healthiest and cheapest sources of animal protein, and it has amino acid compositions that are higher in cysteine than most other sources of protein. The study of heavy metals concentrations in fish tissues, especially muscles, is crucial where it is the main edible part of fish and can directly influence human health (Pintaeva *et al.*, 2011).

This work studied the metals contamination in Nile Tilapia as it is the most familiar and popular fish that represents about 80% of freshwater farmed fishes in Egypt (Emara *et al.*, 2015). The seasonal variations in the concentrations of Pb, Hg, Cd, Zn, Fe, and Cr in muscle tissues of fish samples collected from the Rosetta Branch are shown in Table (3). All metals concentration showed a significant ($P < 0.05$) variations concerning seasons.

The value of Pb was ranged between 0.137 - 1.616 mg kg⁻¹, and it was higher in winter season in Desouk site, while the lowest concentration was recorded in spring season in Kafr El-Zayat site.

The values of Pb were higher than the permissible limit in all sites and seasons except summer and spring seasons. Concerning of Cd concentration in fish muscles, the results showed a wide range of variation between 0.002 - 0.186 mg kg⁻¹, and the highest concentration was observed in autumn season in Kafr El-Zayat site, while the lowest value was detected in spring season in Kafr El-Zayat also. Cd values were higher than the permissible limit in Kafr El-Zayat site and autumn season. Finally; the obtained results showed that the concentration of reminder metals (Hg, Zn, Fe, and Cr) in fish muscles were less than the permissible limit.

These results could be attributed to the impact of seasonal variation on fish organs contents from tested heavy metals, whereas fish live in polluted water tend to accumulate heavy metals in their tissues. In General, the accumulation depends on metals concentration, time of exposure, way of metal uptake, environmental conditions (pH, salinity, hardness, and temperature), and intrinsic factors (feeding habits of fish and age).

These results are in harmony with those obtained by Authman *et al.*, (2013) who found that metal concentrations in fish organs exhibited seasonal variations and they may be due to the increase or decrease of drainage water discharged into the drainage canal. In addition, Barbara and Maágorzata (2006) reported that most of heavy metals concentrations accumulate mainly in liver, kidney, and gills.

Fish muscles, compared to the other tissues; usually contain the lowest levels of metals. In this prospect Okoro *et al.*, (2016) mentioned that heavy metals were more concentrated in the gills than other parts of the fish organs sourced because of relatively high potential for metal accumulation in the gills. Also, Edward *et al.* (2013) reported that the lowest heavy metal concentration in the muscles and the highest one in the gills.

Additionally; Ghanem *et al.*, (2016) declared that the minimum Zn and Fe in fish muscles was measured during summer, and the concentrations are 0.58 ± 0.54 and 4.66 ± 2.46 µg/g for Zn and Fe, respectively. The high concentration of Cd in fish muscles may be associated with the lifestyle of the species that spending more time at the bottom and or muds, (Nziku, 2013).

Table 3: Concentration of heavy metals in muscles fish samples (mg L⁻¹) at the Rosetta Branch during 2016/2017.

Season	Site	The concentration of heavy metals (mg kg ⁻¹)					
		Pb	Hg	Cd	Zn	Fe	Cr
Summer 2016	Kafr El-Zayat	0.158 ^f ±	0.178 ^{ab} ±	0.008 ^c ±	0.008 ^e ±	1.576 ^{cdef} ±	0.433 ^c ±
		0.0137	0.015	0.001	0.005	0.127	0.123
	Minyat Jenaj	0.226 ^f ±	0.102 ^{bcd} ±	0.010 ^e ±	0.009 ^e ±	1.914 ^{cde} ±	0.737 ^b ±
		0.1970	0.051	0.010	0.011	0.192	0.062
	Desouk	0.193 ^f ±	0.049 ^d ±	0.015 ^c ±	0.031 ^e ±	1.900 ^{cde} ±	0.994 ^{ab} ±
		0.0443	0.007	0.008	0.014	0.093	0.277
	Motobas	0.164 ^f ±	0.039 ^d ±	0.010 ^e ±	0.004 ^e ±	1.791 ^{cdef} ±	1.112 ^a ±
0.0315		0.004	0.005	0.001	0.169	0.530	
Mean		0.1851^C±	0.092^B±	0.011^C±	0.013^C±	1.794^B±	0.819^A±
		0.092	0.062	0.007	0.014	0.190	0.378
Autumn 2016	Kafr El-Zayat	0.811 ^{cd} ±	0.045 ^d ±	0.186 ^a ±	0.759 ^d ±	0.094 ^f ±	0.037 ^d ±
		0.0430	0.007	0.010	0.055	0.082	0.020
	Minyat Jenaj	1.020 ^{bcd} ±	0.043 ^d ±	0.145 ^a ±	0.253 ^e ±	0.215 ^{ef} ±	0.027 ^d ±
		0.1951	0.008	0.013	0.060	0.130	0.008
	Desouk	1.131 ^{bc} ±	0.158 ^{abc} ±	0.181 ^a ±	0.605 ^{de} ±	0.271 ^{ef} ±	0.071 ^d ±
		0.2375	0.123	0.014	0.116	0.029	0.041
	Motobas	0.871 ^{bcd} ±	0.120 ^{bcd} ±	0.177 ^a ±	0.737 ^d ±	0.218 ^{ef} ±	0.203 ^{cd} ±
0.3776		0.027	0.045	0.045	0.139	0.040	
Mean		0.958^B±	0.091^B±	0.172^A±	0.589^C±	0.199^C±	0.085^B±
		0.246	0.075	0.027	0.221	0.112	0.078
Winter 2017	Kafr El-Zayat	1.132 ^{bc} ±	0.094 ^{bcd} ±	0.072 ^b ±	2.345 ^a ±	3.970 ^{ab} ±	0.027 ^d ±
		0.268	0.088	0.098	1.512	1.730	0.028
	Minyat Jenaj	0.678 ^{de} ±	0.074 ^{cd} ±	0.075 ^b ±	1.407 ^{bc} ±	0.533 ^{ef} ±	0.044 ^d ±
		0.121	0.013	0.009	0.134	0.202	0.014
	Desouk	1.616 ^a ±	0.122 ^{bcd} ±	0.015 ^c ±	2.632 ^a ±	3.802 ^{ab} ±	0.017 ^d ±
		0.233	0.011	0.001	1.691	0.747	0.011
	Motobas	1.200 ^b ±	0.040 ^d ±	0.010 ^e ±	2.124 ^a ±	4.640 ^a ±	0.014 ^d ±
0.213		0.008	0.001	1.490	0.994	0.002	
Mean		1.157^A±	0.083^B±	0.043^B±	2.627^A±	3.236^A±	0.026^B±
		0.393	0.050	0.053	2.366	1.896	0.019
Spring 2017	Kafr El-Zayat	0.137 ^f ±	0.226 ^a ±	0.002 ^c ±	1.955 ^b ±	2.447 ^{bcd} ±	0.028 ^d ±
		0.157	0.049	0.003	0.831	0.295	0.033
	Minyat Jenaj	0.335 ^{ef} ±	0.106 ^{bcd} ±	0.005 ^e ±	2.560 ^a ±	0.852 ^{def} ±	0.060 ^d ±
		0.047	0.020	0.009	0.326	0.404	0.026
	Desouk	0.428 ^{ef} ±	0.125 ^{bcd} ±	0.018 ^c ±	2.712 ^a ±	1.397 ^{cdef} ±	0.050 ^d ±
		0.088	0.029	0.003	0.638	0.393	0.026
	Motobas	0.368 ^{ef} ±	0.107 ^{bcd} ±	0.007 ^c ±	2.167 ^a ±	2.645 ^{bc} ±	0.042 ^d ±
0.304		0.014	0.008	0.350	2.881	0.015	
Mean		0.3170^C±	0.141^A±	0.008^C±	2.348^B±	1.835^B±	0.045^B±
		0.190	0.058	0.008	0.584	1.476	0.025
All over mean	Kafr El-Zayat	0.559^b±	0.136^a±	0.067±	2.767^a±	2.021^a±	0.131^b±
	Minyat Jenaj	0.565^b±	0.081^b±	0.059±	1.057^b±	0.878^b±	0.217^{ab}±
	Desouk	0.842^a±	0.114^{ab}±	0.057±	2.995^a±	1.841^a±	0.283^a±
	Motobas	0.651^b±	0.077^b±	0.051±	2.758^a±	2.324^a±	0.343^a±
	Mean	0.483	0.042	0.078	2.399	1.594	0.522
* permissible limits		0.5	0.5	0.05	40	30	5.5
** permissible limits		0.2	0.5	0.05	100	100	NA

* EOSQC, Egyptian Organization for Standardization and Quality Control (1993 and 2005).

** FAO/WHO (1989) and FAO, (2003). NA = not available

- Means values (mean ± SD, n = 3) within a column with a same superscript are not significantly different (P > 0.05)

Evaluation of correlation between heavy metal contents in water, gills, and muscles

The relationship among concentrations of heavy metals in water, gills, and muscles were presented in Table (4). There was a highly significant positive correlation between Pb concentration in water and concentration of Cd and Cr in water, gills, and muscles. Also, there are a positive relationship between Pb in water and Pb in gills and muscles. Besides, there was a significant positive correlation between Pb in water and concentration of Fe and Hg in water and gills. On the other hand, there was a significant negative correlation between Pb in muscles and Cr in muscles.

Table 4: Correlation coefficient values between heavy metal contents in water, gills, and muscles.

Metals	Pb water	Cd water	Hg water	Cr water	Fe water	Zn water	Pb gills	Cd gills	Hg gills	Cr gills	Fe gills	Zn gills	Pb Muscle	Cd Muscle	Hg Muscle	Cr Muscle	Fe Muscle	Zn Muscle	
Pb water	1																		
Cd water	0.89**	1																	
Hg water	0.39	0.41	1																
Cr water	0.82**	0.92**	0.45	1															
Fe water	0.72**	0.77**	0.06	0.84**	1														
Zn water	0.13	-0.12	0.53*	-0.01	-0.19	1													
Pb gills	0.69**	-0.04	0.19	-0.54*	0.37	0.69**	1												
Cd gills	0.80**	0.31	0.13	-0.46	0.18	0.39	0.36	1											
Hg gills	0.60*	0.37	0.32	-0.66**	0.26	0.32	0.39	0.08	1										
Cr gills	0.84**	0.31	0.09	-0.49	0.18	0.41	-0.62**	-0.27	-0.57*	1									
Fe gills	0.61*	0.11	-0.31	-0.35	0.27	0.45	0.18	-0.44	0.03	-0.29	1								
Zn gills	0.16	-0.29	0.50*	-0.53*	0.47	0.55*	0.42	-0.52*	0.24	-0.51*	0.71**	1							
Pb Muscle	0.71**	0.06	-0.21	-0.61*	0.42	0.58*	0.84**	0.52*	-0.13	-0.69**	0.06	0.36	1						
Cd Muscle	0.86**	0.42	-0.28	-0.52*	0.19	0.24	0.30	0.83**	-0.15	-0.13	-0.62*	-0.46	0.46	1					
Hg Muscle	0.47	0.42	0.07	-0.71**	-0.12	0.39	0.07	0.15	0.42	-0.55*	-0.29	0.46	-0.19	-0.15	1				
Cr Muscle	0.86**	0.48	-0.27	-0.55*	0.18	0.30	-0.51*	-0.30	-0.29	0.93**	-0.02	-0.61*	-0.52*	-0.31	-0.28	1			
Fe Muscle	0.80**	0.21	-0.45	-0.36	0.44	0.22	0.24	-0.39	0.46	-0.34	0.64**	0.57*	0.24	-0.63**	0.01	-0.04	1		
Zn Muscle	-0.02	-0.13	0.39	-0.59*	0.14	0.74**	0.48	-0.41	0.13	-0.58*	0.73**	0.92**	0.32	-0.31	0.22	-0.68**	0.48	1	

*Significant

**High significant

Positive correlation with highly significant was observed between Cd concentration in water and concentration of Cr and Fe in water. Also, there was a significant negative correlation between Cd in gills and Cr in gills and muscles, but it was highly positive significant with Pb in muscles. In addition, there was a highly significant negative correlation between Cd in muscles and Fe in muscles.

Regarding with correlation between Hg concentration in water and other elements, the positive correlation was mentioned with Zn concentration in water, gills, and muscles only. Also, there was a significant negative correlation between Hg in gills and Cr in gills. Data also showed that there was a highly significant positive correlation between Cr in water and Fe in water, and it was a highly significant negative correlation with Hg in gills and muscles, but it was negative with Pb and Zn in gills and muscles, Cd and Cr in muscles only. Moreover, there was a highly positive significant correlation between Cr in gills and muscles. On the other hand, the highly significant negative correlation was observed between Cr in gills and Pb in muscles, but it was significant with Zn in gills, muscle, and Hg in muscles only. Finally, the highly significant negative correlation was detected between Cr and Zn in muscles. Concerning with the relationship between Fe concentration and other elements, the obtained data indicated that there was a highly significant positive

correlation between Fe and Zn in gills and muscles also with Fe in muscles. In vice versa, it was negative with Cd in muscles. Finally; the results of correlation coefficient showed that there was a highly significant positive correlation between Zn in gills and muscles, and it was significant with Fe in muscles, but it was vice versa with Cr in muscles.

It could be concluded that the concentration of all heavy metals (Pb, Cd, Hg Fe, Zn, and Cr) in fish gills was highest followed by muscles and water in all seasons and sites. These results are an agreement in part with those obtained by Afiukwa (2013) who indicated that a significant positive correlation between Pb/Zn, Cd/Zn, and Fe/Pb in drinking water. While chromium gives negative correlation with the other metals, Also, Emara *et al.*, (2015) found that significant positive correlations between (Fe and Cd; Cd and Pb) in fish muscle tissues. The correlations between the different metals may result from the similar accumulation behavior of the metals or that share the same chemical environment or absorptive pathways. The presence of such metals in association affects the occurrence and absorption of the other metals. On the other hand, metals which give the negative correlation with the other metals may be due to different sources of the metal.

Effect of heavy metals on indicator growth of Nile Tilapia fish

The results in Table (5) showed that there were significant differences of seasonal variation on indicator growth of Nile tilapia fish which grow in the freshwater of the Rosetta Branch during the year of 2016/2017. The winter season was recorded the highest value of fish length and weight with the mean values of 19.5 cm and 153.1 g, respectively, while the autumn season was recorded the lowest values of 15.5 cm and 80.5 g for both parameters.

Table 5: Effect of heavy metals on growth of Nile tilapia fish

Season	Site	Length (cm)	Weight (g)	CF*	HSI**
Summer 2016	Kafer El zayat	18.9 ^{cd} ±0.85	137.2 ^c ±23.1	2.00 ^{bcd} ±0.15	1.17 ^{cdef} ±0.23
	Minyat Jenaj	17.5 ^{de} ±1.41	100.9 ^{def} ±20.0	1.86 ^{cd} ±0.20	0.67 ^f ±0.45
	Desouk	16.4 ^{efg} ±0.09	116.9 ^{cde} ±29.3	2.66 ^a ±0.63	0.86 ^{ef} ±0.52
	Motobas	16.5 ^{ef} ±0.79	94.8 ^d ±11.5	2.13 ^{bc} ±0.39	0.61 ^f ±0.36
	Mean	17.3^B±1.4	112.4^B±26.2	2.16^A±0.48	0.83^C±0.43
Autumn 2016	Kafer El zayat	16.3 ^{efg} ±0.44	101.6 ^{def} ±9.2	2.35 ^{ab} ±0.23	1.75 ^{ab} ±0.65
	Minyat Jenaj	15.5 ^{fg} ±0.79	77.6 ^{fg} ±10.8	2.07 ^{bc} ±0.07	1.80 ^{ab} ±0.36
	Desouk	14.7 ^g ±0.83	62.6 ^g ±8.9	1.97 ^{bcd} ±0.24	0.59 ^f ±0.39
	Motobas	15.6 ^{fg} ±0.96	80.2 ^{fg} ±9.5	2.13 ^{bc} ±0.32	0.60 ^f ±0.40
	Mean	15.5^C±0.9	80.5^C±16.8	2.13^A±0.26	1.19^B±0.73
Winter 2017	Kafer El-Zayat	21.2 ^b ±0.57	192.2 ^b ±24.8	2.01 ^{bcd} ±0.12	0.91 ^{def} ±0.35
	Minyat Jenaj	16.2 ^{efg} ±1.09	80.6 ^{efg} ±13.0	1.78 ^{cd} ±0.48	0.85 ^{ef} ±0.25
	Desouk	16.2 ^{efg} ±1.44	70.2 ^{fg} ±15.0	1.65 ^d ±0.20	0.74 ^{ef} ±0.32
	Motobas	24.6 ^a ±2.19	269.4 ^a ±63.0	1.79 ^{cd} ±0.15	1.28 ^{bcd} ±0.42
	Mean	19.5^A±3.9	153.1^A±90.6	1.80^B±0.29	0.94^{BC}±0.38
Spring 2017	Kafer El zayat	17.8 ^{cde} ±1.09	106.0 ^{cdef} ±14.0	1.88 ^{cd} ±0.19	1.85 ^a ±0.37
	Minyat Jenaj	17.2 ^{ef} ±1.78	99.4 ^{def} ±32.6	1.90 ^{cd} ±0.22	1.71 ^{abc} ±0.45
	Desouk	17.2 ^{ef} ±0.83	101.0 ^{def} ±10.8	1.98 ^{bcd} ±0.10	1.46 ^{abcd} ±0.28
	Motobas	19.3 ^c ±2.04	130.0 ^{de} ±33.5	1.79 ^{cd} ±0.12	1.17 ^{cdef} ±0.26
	Mean	17.9^B±1.65	109.1^B±26.2	1.89^B±0.17	1.55^A±0.42
All over mean	Kafer El zayat	18.57 ^b ±1.97	134.2 ^a ±40.9	2.06±0.24	1.42 ^a ±0.56
	Minyat Jenaj	16.61 ^c ±1.47	89.6 ^b ±22.1	1.91±0.28	1.26 ^a ±0.63
	Desouk	16.12 ^c ±1.26	87.7 ^b ±28.1	2.07±0.50	0.92 ^b ±0.49
	Motobas	19.00 ^a ±3.89	143.6 ^a ±83.8	1.96±0.30	0.92 ^b ±0.46

* = Condition factor ** = Hepato-somatic index

- Means values (mean ± SD, n = 10) within a column with a same superscript are not significantly different (P > 0.05)

Concerning with sampling site, the obtained results showed that the Motobas site was detected the highest fish length and weight with mean values of 19.0 cm and 143.6 g, respectively while the Desouk site was detected the lowest values of 16.12 cm and 87.7 g for both parameters. The interaction between seasons and sites revealed that the highest fish length and weight was noticed in Motobas site during winter season with the mean value of 24.6 cm and 269.4 g for both parameters, respectively. The lowest values were observed in Desouk during autumn season with the mean values of 14.7 cm and 62.6 g for both parameters, respectively. These results could be attributed to the fluctuation of water level and temperature that determine the body condition of fish. These results are in harmony with those obtained by Otieno *et al.*, (2014) who noted that the changes in the availability of food items and water quality, and fluctuation of water level and water temperature that determine the body condition of fishes. On the other hand the results are disagreement with those obtained by Abdelhamid *et al.*, (2013) who indicated that autumn season gave the best fish weight and total length of Nile Tilapia fish in the River Nile at Helwan area, and it could be attributed to the variation of geographic location between Helwan and Rosetta Branch.

In addition, the obtained results showed that the growing season has the significant impacts on condition factor (CF) of Nile Tilapia fish (Table 5), and the summer season was recorded the highest value of CF with the mean value of 2.16, while the lowest value (1.8) was recorded in winter season. Also, the obtained data revealed that there was an insignificant difference between sites on CF. Regarding the interaction between seasons and sites, the highest CF was observed in Desouk site during the summer season, and the lowest CF was recorded in the same site during the winter season. Additionally, the mean hepato-somatic index (HSI) for Nile Tilapia fish ranged between 0.95 and 1.58. The spring season was detected the highest value (1.55), while the lowest value (0.83) was recorded in the summer season. The sites also have significant differences on HSI and Kafer El-zayat was given the highest value followed by Minyat Jenaj, Desouk, and Motobas with mean values of 1.42, 1.26, 0.92, and 0.92, respectively. The interaction between seasons and sites indicated that the highest value (1.85) of HSI was noticed in Kafer El-zayat during the spring season, while the lowest value (0.59) was observed in Desouk during the autumn season. In this prospect, CF and HSI are essential biological parameters used to assess the fish condition concerning the feeding condition. The length-weight relationship is significant for proper exploitation and management of the population of fish species (Anene, 2005). Also, condition factor decreases with an increase of length, whereas the CF value is higher, it means that the fish has attained a better condition. The results obtained from the present study could be attributed to that the fish condition could be affected by water quality and availability of food items. The results obtained from the present study are in agreement with those obtained by Khallaf *et al.*, (2014) and Agumassie (2018) they reported that the fish condition was affected by the number of factors such as stress, sex, season, availability of feeds, and water quality. Besides, the change of HSI value could be attributed to the metal's toxicity on fish, whereas it is a good indication of impaired reproductive physiology. Also, the size of the liver was varied according to climate conditions, as a result of the consequence of a loss of hepatic glycogen and/or lipid which is a typical morphologic response of the fish liver to environmental stress, (Saeed, 2013).

CONCLUSION

It can be concluded that the seasonal variations and geographic locations had significant impacts on water and fish quality at the Rosetta branch. The winter was the highest season in heavy metals concentration in water and fish samples, and summer was the lowest one. Additionally; Kafr El-Zayat was the highest site in heavy metals concentration, and Minyat Jenaj was the lowest one. Pb and Hg concentrations in the water were higher than the permissible limit. So, efforts should be directed towards the protection of the River Nile from pollution through treatment of wastewater and from pollutant before entrance to the Nile River or stopping the discharge these pollutants into the Nile River to protect fish and human from the deleterious effects of pollution.

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ARABIC SUMMARY

الاختلافات الموسمية للمعادن الثقيلة في مياه وأسمك البلطي النيلي في فرع رشيد

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يعد تلوث البيئة المائية بالمعادن الثقيلة أحد أهم مشكلات المياه والحياة المائية والصحة العامة في فرع رشيد. هناك زيادة سنوية في الملوثات والمعادن الثقيلة التي تتدفق في نهر النيل من قبل العديد من المصادر مثل الأنشطة الزراعية والصناعية ومياه الصرف. وهدفت الدراسة الحالية إلى تقييم الاختلافات الموسمية لمحتويات المعادن الثقيلة (الرصاص والكاديوم والزنك والكروم والحديد والزنك) في عينات الماء وأسمك البلطي النيلي التي تم جمعها من فرع رشيد ومعرفة مدى تأثيرها على نمو الأسماك وجودة المياه. وقد أجريت هذه الدراسة في أربعة مواقع (كفر الزيات، منية جناح، دسوق، مطوبس) على طول فرع رشيد خلال عام ٢٠١٧/٢٠١٦ حيث تم جمع العينات خلال أربعة مواسم (الصيف والخريف والشتاء والربيع). أظهرت نتائج الدراسة أن موسم الشتاء سجل أعلى تركيز للمعادن الثقيلة في المياه بينما كانت التركيزات الأقل خلال موسم الصيف. بالإضافة إلى ذلك أعطى موقع كفر الزيات أعلى تركيزات للمعادن الثقيلة بينما سجلت منية جناح أقل تركيز للمعادن الثقيلة. سجل عنصر الزنك في الماء أعلى التركيزات وكانت أعلى من الحدود المسموح بها في كل المواقع والمواسم. أيضا سجل الرصاص نفس الاتجاه في كل المواقع والمواسم باستثناء موسم الصيف. وبالنسبة لتركيز الكاديوم كان أعلى من الحدود المسموح بها في موسمي الخريف والشتاء. بالنسبة لعينات الأسماك فكانت تركيزات المعادن الثقيلة في الخياشيم أعلى بكثير من عضلات الأسماك. في موقعي دسوق ومطوبس كان تركيز الرصاص في العضلات أعلى من الحد المسموح به وفي موسمي الخريف والشتاء. كان تركيز الكاديوم في العضلات أعلى من الحدود المسموح بها في موسم الخريف في منطقة كفر الزيات، وأعطى موسم الربيع أعلى القيم لمؤشر الكبد الجسدي مقارنة بمواسم الدراسة الأخرى.