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Occurrence and human health risks of pesticides and antibiotics in Nile tilapia along the Rosetta Nile branch, Egypt

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) samples were collected at monthly intervals between July 2018 and June 2019 from 3 sampling sites (El-Rahawy, Sabal and Tala) situated along the Rosetta branch of the Nile, Egypt, to monitor the presence of 100 pesticides and 5 antibiotics using different extraction procedures followed by GC–MS/MS and LC–MS/MS. Potential human health risks via the consumption of contaminated fish was also assessed. Of the 72 analyzed fish muscle samples; 86% and 21% were contaminated with pesticides and antibiotics, respectively. Chlorpyrifos (ranging from < LOQ to 0.08 mg/kg) was the most frequently detected pesticide followed by *p,p′*-DDE (ranging from < LOQ to 0.04 mg/kg) in 83 and 45% of the fish muscle samples, respectively. Nitrofurazone (ranging from 8.6 to 52 μg/kg) was the most frequently detected antibiotic, followed by nitrofurantoin (ranging from 1.1 to 2 μg/kg) and chloramphenicol (ranging from < LOQ to 0.17 μg/kg). These antibiotics were found in 12, 6 and 5% of the fish muscle samples, respectively. The spatial distribution of the detected pesticides and antibiotics in fish samples along the Rosetta branch showed that the highest mean concentrations were found in the Sabal area, followed by samples from Tala and El-Rahawy. An investigation into seasonal variations revealed that the highest mean concentrations of pesticides and antibiotics in fish samples were detected in winter and spring, respectively. According to target hazard quotient (THQ) calculations for the detected pollutants in Nile tilapia muscle, all pollutants gave THQ values lower than 1, indicating that the consumption of this fish from the study sites is unlikely to cause any detrimental effects to consumers.

1. Introduction

The River Nile separates at Cairo into the Rosetta and Damietta branches, both of which form the Nile delta. Demand on water resources on the Rosetta branch for irrigation, domestic water supply, and industrial uses is continuously increasing due to population growth, expanding urbanization, industrialization, and agriculture [1]. At the same time, its water quality is deteriorating due to domestic plus industrial wastewater inputs and agricultural run-off [2]. Therefore, pollution is a pressing issue, with consequences for both environmental and human health.

Monitoring the Rosetta Nile branch's pollutants is necessary for providing quantitative data on the most abundant organic micropollutants, to identify their sources, for understanding their fate, regulatory compliance, human and ecological health implications, and to enable advanced treatment technologies to be better targeted. This lack of baseline knowledge for the river Nile on pollution and its effects hampers the development of management plans and the application of novel remediation technologies.

Pollutants in the waters of the Nile not only represent a threat to human health through contamination of the water and food supply, but also to aquatic organisms in general. Fish are one of the most affected species, as they are exposed to the pollutants in the aquatic environment through their skin, gills, and diet [3]. In fact, water pollution is the most important factor affecting the quality of fish production in its natural habitat, since the pollutants can end up in the tissue of aquatic organisms and subsequently bioaccumulate in the fish over time [4]. Fish consumption can be therefore considered as one of the major sources of human exposure to certain environmental contaminants. Nile tilapia (*Oreochromis niloticus*) has a wide distribution in the Nile, and is widely consumed in Egypt. Consequently, its contamination is a matter of concern for human health.

The occurrence of pesticides in water bodies is of great concern owing to their toxicity and environmental persistence. They also can accumulate significantly in fish tissues through the food chain and pose a severe threat to human health [5]. Nowadays, antibiotics are used

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extensively for treatment and prevention of human and animal diseases; and also, as growth promoters in animal production. Generally, antibiotics are released into the aquatic environment via sewage treatment plant effluent, improper disposal of expired products, hospital waste, manufacturing plant waste, runoff from intensive agricultural operations, and excreta from both human and animals [6]. Therefore, the occurrence and potential environmental impact of their residues have increasingly received attention worldwide [7,8]. Previous studies in the river Nile have mainly been limited to focusing on a small number of pesticides and never targeted antibiotics [9,10].

Chronic intake of contaminants above their safe threshold in humans can have detrimental effects and can cause non-carcinogenic hazards such as neurologic alterations and hepatorenal dysfunction [11]. The target hazard quotient (THQ) set by the United States Environmental Protection Agency (US EPA) is commonly used to estimate the potential non-carcinogenic health risks associated with long term exposure to a variety of contaminants via fish consumption [12].

In this context, the objectives of this study were: i) to monitor the occurrence of 100 pesticides and 5 antibiotics in Nile tilapia samples along the Rosetta branch of the river Nile, Egypt and ii) to assess the potential human health risks for the local population through a life time consumption of contaminated Nile tilapia from the Rosetta branch of the Nile. As far as the authors know, this is the first study that simultaneously monitors the presence of such an extensive number of contaminants in the river Nile, especially antibiotics as emerging contaminants, at monthly intervals for one year using advanced analytical instruments.

2. Materials and methods

2.1. Study area and sampling

Along the Rosetta branch of the Nile, a total of 72 wild Nile tilapia samples (*Oreochromis niloticus*) were obtained in duplicate from 3 sampling sites as follows: 1 Km before the outlet of (**1**) the El-Rahawy drain (Giza governorate; coordinates of 30°12′25.41″N and 31° 1′48.35″E), (**2**) the Sabal drain (Minoufiya governorate; coordinates of 30°31′57.94″N and 30°50′53.20″E) and (**3**) the Tala drain (Kafr El-Zayat, Gharbiya governorate; coordinates of 30°48′58.19″N and 30°48′37.60″E) as shown in Fig. 1.

Fish sampling campaigns were carried out at all sites monthly from July 2018 to June 2019 to monitor the presence of 100 pesticides and 5 antibiotics. The full list of the pesticides and antibiotics selected as target contaminants and their percentage recoveries and coefficient of variation at different standard levels and limits of quantification (LOQ) are provided in the Supplementary Material (Table S1-2).

Samples were collected from each site (2 kg) with the help of local fishermen and immediately transferred to the Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Dokki, Giza in an icebox. Upon arrival fish samples were washed thoroughly with tap water to remove any adhering contaminants then the edible parts of each fish were obtained from the dorsal muscle tissues, homogenized and kept at −20 °C until analysis.

2.2. Pesticide residues analysis

The extraction of pesticide residues was performed by weighing 10 ± 0.1 *g* of fish into a 50-ml polypropylene centrifuge tube. Ten mL of acetonitrile was added and the samples shaken vigorously for a minute, homogenized using an Ultra-Turrax for 2 min at high speed (15,000 rpm) and then extraction kits based on the quick, easy, cheap, effective, rugged, and safe (QuEChERS) methodology added [13]. The tube was shaken vigorously for 1 min, and centrifuged for 5 min at 2812 *g*. About 1.0 mL of the supernatant portion was filtered and transferred into a vial for liquid chromatography-tandem mass spectrometry (LC/MS-MS) analysis. Five mL of the remaining supernatant was transferred into another 15 mL tube containing 900 mg ($MgSO_4$) and 150 mg primary secondary amine (PSA) for dispersive solid-phase extraction (DSPE). The tube was shaken vigorously for 1 min and then centrifuged at 2812 *g* for 2 min. Two mL of the supernatant was taken and evaporated to dryness. The samples were reconstituted using 2 mL n-hexane: acetone (9:1 v/v) containing 0.1 mg/kg of aldrin as an internal standard, followed by ultra-sonication for 30 s. Finally, the sample was filtered into a vial for gas chromatography-tandem mass spectrometry (GC/MS-MS) analysis.

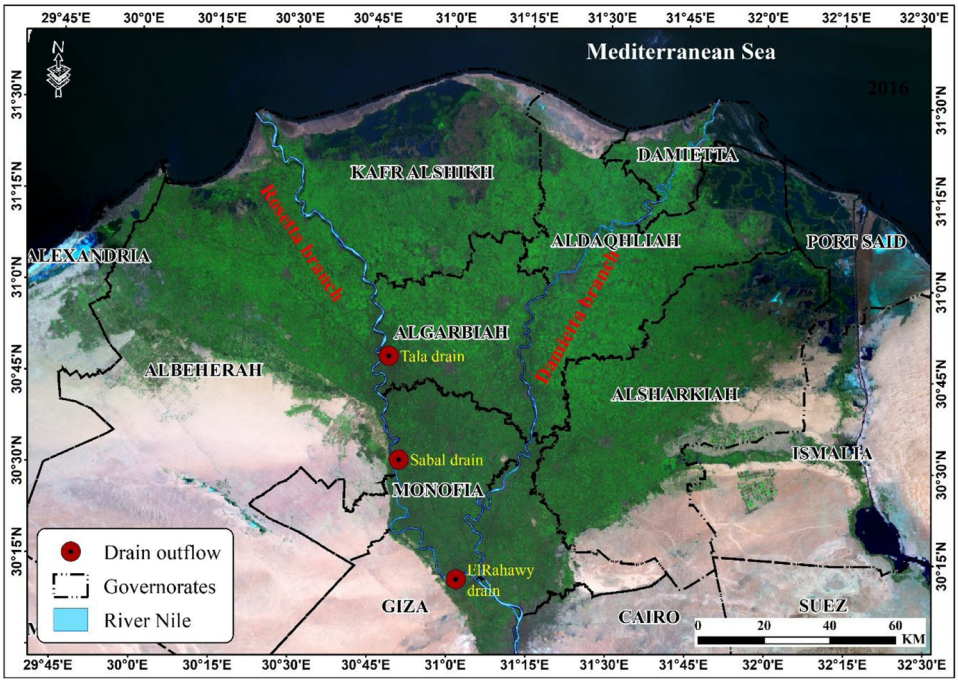

Fig. 1. The sampling sites of Nile tilapia along the Rosetta branch, river Nile, Egypt.

2.3. Chloramphenicol analysis

Ten mL of acetonitrile was added to 1 *g* of the previously homogenized sample. One mL of 1 M citric acid (pH 4.0) and 0.5 mL of 0.5 M EDTA–Na_2 solution were added and the sample homogenized for 2–3 min using an Ultra Turrax and then shaken for one minute. The sample was centrifuged at 2222 *g* for 10 min and the supernatant was evaporated under a stream of nitrogen and taken up in 1 mL of the mobile phase, before injecting 20 µL into the LC-MS/MS system [14].

2.4. Nitrofuran metabolites analysis

One g of the previously homogenized tissue sample was weighed into a 50 mL plastic tube then 4 mL de-ionized water was added to the sample followed by 0.5 mL of 1 M HCl and 150 µl of 50 mM methanolic 2-nitrobenzaldehyde. The tube was capped, vortexed for 10 s and finally incubated at 55 °C in an agitated water bath for 4 h. The solution was then neutralized by adding 5 mL of 0.1 M potassium dihydrogen phosphate, followed by 300 µl of 1 M NaOH. The tube was swirled for a few seconds and the pH was checked to ensure it was at 7.0 ± 0.5 with pH strips. Liquid-liquid extraction was performed by adding 5 mL ethyl acetate to the neutralized solution and mixing for 20 min on a rotary homogenizer. The ethyl acetate portion was then extracted by centrifugation at 3000 *g* for 10 min at 4 ± 1 °C, and the supernatant was transferred into a clean 15 mL disposable polypropylene tube. The neutralized solution was re-extracted with 3 mL of ethyl acetate and mixed for 20 min. Centrifugation at 3000 *g* for 10 min at 4 ± 1 ∘C was carried out, the new supernatant was transferred into the same 15 mL disposable polypropylene tube to give a total volume of 8 mL ethyl acetate volume containing the nitrophenyl derivatized residues of the nitrofuran metabolites. The solution was evaporated at 45 °C under a gentle stream of nitrogen to near dryness, and re-dissolved in 400 µl dilution solvent consisting of methanol/1 mM ammonium formate (60/40; v/v), before placing for 1 min in water ultra-sonication bath and then ultra-centrifuging at 19,200 *g* for 20 min at 4 ± 1 °C. The supernatant was then filtered through a disposable 0.45 µm acrodisc and the filtrate was transferred into a vial for LC–MS/MS analysis [15].

2.5. GC–MS/MS analysis

An Agilent 7890A gas chromatography system equipped with a 7000B triple quadrupole Agilent mass spectrometer was used. The column was DB-35 MS Ultra inert capillary column (35% Phenyl-65% dimethylpolysiloxane, 30 m length ×0.18 mm i.d. × 0.25 µm film thickness, Agilent Technologies). The oven temperature program was held at 70 °C for 1.3 min, and then ramped at 70 °C/min up to 150 °C, then at 12 °C/min up to 270 °C, and finally at 18 °C/min up to 310 °C, which was held for 6.3 min giving a total run time of 21 min. The inlet temperature was 250 °C and the injection volume was 1 µL injected in splitless mode. Helium was used as the carrier gas at a constant flow rate of 0.7 ml/min and nitrogen used as the collision gas. Electron impact mode was used and the ionization energy was 70 eV, the ion source temperature was 320 °C, the GC–MS/MS interface temperature was 320 °C and the Quadrapole temperature was 180 °C. MassHunter softwares were used for instrument control and data acquisition/processing.

2.6. LC–MS/MS analysis

The LC–MS/MS system consisted of an Agilent 1200 Series HPLC linked to an API 4000 Qtrap MS/MS from Applied Biosystems (Foster City, CA, USA) was used. The separation was performed on an Agilent C18 column ZORBAX Eclipse XDB with 150 mm length, 4.6 mm i.d. and 5.0 µm particle size. The column temperature was 40 °C and the injection volume was 5 µL. The separation was done using gradient elution between two components, A: 10 mM ammonium formate solution in methanol: water (1:9 v/v) and B: methanol. The initial flow rate was 0.5 ml/min starting with 100% of component A and gradually changing to 5% A (95% B) over 6 min, which was kept constant for 17 min at a flow rate of 0.3 ml/min. After this 23 min run time, 2 min of post-time followed using the initial 100% of A at a flow rate of 0.5 ml/min. The MS/MS analysis was done by electrospray ionization (ESI) in the positive ion mode with multiple reaction monitoring mode (MRM). The following source and gas parameters were applied: temperature, 450 °C; curtain gas, 25 psi; collision gas, medium; ion spray voltage, 5000 V; ion source gas 1, 40 psi; and ion source gas 2, 40 psi. Analyst software 1.6. was employed for instrument control and data acquisition/processing.

2.7. Quality assurance

All analytical methods and instruments were fully validated as part of a laboratory quality assurance system and were audited and accredited by the Centre for Metrology and Accreditation, Finnish Accreditation Service (FINAS), Helsinki, Finland. This quality system is referred to as ISO/IEC 17,025:2005. The blank and spiked samples were analyzed according to the aforementioned methods to calculate the extraction efficiency. The average recoveries of the selected pesticides and antibiotics in fish samples varied between 62–120% and 85–97%, respectively. The limits of quantification (LOQ) were 0.01 - 0.05 mg/kg and 0.075 - 0.5 µg/kg for pesticide residues and antibiotics, respectively. These are shown in Table S1-2 (Supplementary Material).

2.8. Human risk assessment

The potential non-carcinogenic health risks associated with the consumption of Nile tilapia containing contaminants were estimated based on the target hazard quotient (THQ) which was calculated using the following equation [12]:

$$THQ = (EF \times ED \times FIR \times C) / (RfD \times BW \times AT)$$

where EF is exposure frequency (365 days/year), ED is the exposure duration (70 years; equivalent to the average human lifetime), FIR is the fish ingestion rate (38.14 g/person/day) [16], C is the contaminant concentration in Nile tilapia (mg/kg, wet weight), RfD is the oral reference dose [17] (presented in Tables 3–4), BW is the body weight (60 kg/person), AT is the averaging time for non-carcinogens (365 days/year × ED). When the THQ is < 1, there is no obvious health risk for the exposed population. The population will experience health risks when THQ is ≥1, and interventions and protective measures should be then taken [18].

3. Results and discussion

3.1. Spatial and temporal distribution of contaminants in Nile tilapia along the Rosetta Nile branch

3.1.1. Pesticide residues

As shown in (Table 1), of the 72 analyzed Nile tilapia muscle samples; 62 were contaminated with one or more pesticide residues. The spatial distribution of the detected pesticides in fish muscle samples along the Rosetta branch showed that Sabal registered the highest sum of all pesticide mean concentrations (0.126 mg/Kg), followed by Talal (0.075 mg/Kg) and El-Rahawy (0.022 mg/Kg). The Sabal site was contaminated with 6 pesticides. On the contrary, Tala and El-Rahawy sites were contaminated with 4 and 2 pesticides, respectively. Pesticides may be transferred to water bodies through surface run-off, erosion, spray drift, leaching and drain flows.

With regard to pesticides per category, insecticides were the most frequently detected pesticides (86%), followed by acaricides (8%), whereas both fungicides and herbicides were similar (3%). Among the

Table 1
Spatiotemporal distribution of pesticide residues (mg/kg ww) in Nile tilapia muscle samples collected from the Rosetta branch of the river Nile, Egypt.

Pesticide		Summer 2018			Autumn 2018			Winter 2019			Spring 2019		
		El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala
Contaminated samples %		83 %	100 %	100 %	100 %	100 %	100 %	57 %	100 %	50 %	25 %	100 %	100 %
Atrazine	Conc. range (mg/kg)	BDL[b]	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BQL-0.01	BDL
	Mean[a] (mg/kg)	–	–	–	–	–	–	–	–	–	–	0.01	–
	Freq. %	–	–	–	–	–	–	–	–	–	–	25 %	–
Chlorpyrifos	Conc. range	0.01	0.01 - 0.07	BQL-0.05	BQL-0.02	0.03 - 0.08	0.02 - 0.05	BQL-0.02	0.01 - 0.03	0.02 - 0.04	0.01	0.01 - 0.08	BQL-0.02
	Mean	0.01	0.03	0.03	0.0125	0.05	0.03	0.015	0.02	0.03	0.01	0.026	0.013
	Freq. %	67 %	100 %	100 %	100 %	100 %	100 %	43 %	100 %	50 %	25 %	100 %	86 %
Fludioxonil	Conc. range	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.03	BDL	BDL	BDL	0.01
	Mean	–	–	–	–	–	–	–	0.03	–	–	–	0.01
	Freq. %	–	–	–	–	–	–	–	14 %	–	–	–	14 %
o,p′- DDT	Conc. range	BDL	BDL	0.01	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Mean	–	–	0.01	–	–	–	–	–	–	–	–	–
	Freq. %	–	–	33%	–	–	–	–	–	–	–	–	–
pp′- DDE	Conc. range	BQL[c]	BQL-0.01	0.02 - 0.04	BDL	BQL	BDL	BQL -0.01	BQL-0.02	BDL	BDL	BQL-0.02	BQL-0.01
	Mean	BQL	0.01	0.03	–	BQL	–	0.01	0.012	–	–	0.012	0.01
	Freq. %	33%	33 %	33 %	–	33 %	–	43 %	100 %	–	–	88 %	86 %
Propiconazole	Conc. range	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02	BDL	BDL	BDL	BDL
	Mean	–	–	–	–	–	–	–	0.02	–	–	–	–
	Freq. %	–	–	–	–	–	–	–	14%	–	–	–	–
Tebufenpyrad	Conc. range	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.01 - 0.03	BDL	BDL	BDL	BDL
	Mean	–	–	–	–	–	–	–	0.016	–	–	–	–
	Freq. %	–	–	–	–	–	–	–	71%	–	–	–	–

[a] The mean concentration of each season or sampling site represents the mean value of three months.
[b] BDL:Below detection limit.
[c] BQL:Below quantification limit.

insecticides, organophosphorus pesticides were the most frequently detected group in fish samples (83%) followed by organochlorines (58%). The most frequently detected pesticides were chlorpyrifos (83%) followed by *p,p′*-DDE (45%) and tebufenpyrad (8%). Atrazine, fludioxonil and *o,p′*-DDT all exhibited the same frequency% (3%). Propiconazole appeared to be the lowest encountered pesticide in all fish samples (2%). The levels of chlorpyrifos exceeded the maximum residue limit (MRL, 0.01 mg/kg, EU pesticides data base) in 45% of the examined samples. Out of these samples, 63, 30 and 7% were found at Sabal, Tala, and El-Rahawy, respectively.

These findings indicate the continuous and intensive use of chlorpyrifos throughout the year since it was found at all sampling sites and in all seasons. These results are in partial agreement with those obtained by Khallaf et al. [9], who found chlorpyrifos residues at 0.504 mg/kg ww only during the spring season in tilapia fish muscle samples collected from Bahr Shebeen Canal, a river Nile Canal, from September 2014 to December 2015. However, Shalaby et al. [10] found chlorpyrifos residues at concentrations that ranged from 8.7 to 11.8 μg/kg ww only during the autumn season in tilapia fish muscle samples collected during 2014–2015 from the Nile River in the Cairo region. Tilapia fish sampled from Alamo River, California, USA, had measurable concentrations of chlorpyrifos in fillets, suggesting continued exposure in the water column to chlorpyrifos [19].

The occurrence of pesticides in fish was closely related to their properties (i.e., the octanol-water partition coefficient (log K_{ow}) and environmental persistence) as well as the intensity of application of particular pesticides [20]. Log K_{ow} is often used as an indicator of the lipophilicity and bioaccumulation potential of pesticides in living organisms. All the detected pesticides, except for atrazine, had **log** K_{ow} values ranging from 3.72 to 6.91. This implies that they have a high bioaccumulation potential in aquatic biota [21].

As for the seasonal variation of detected pesticide residues in fish muscle samples when summing their mean concentrations, it was observed that pesticide residues were higher in the winter season (0.098 mg/kg) compared to summer (0.057 mg/kg), spring (0.053 mg/kg) and autumn (0.028 mg/kg). The seasonal differences in the levels of pesticides may be related to the flow conditions of the river system and their use patterns by the neighboring activities [22]. In general, the water level and flow of the Rosetta branch are lower in winter owing to the closure period, and consequently, the concentration of pesticides is expected to be higher than in the summer season. This is also probably due to the cold-water temperature, which has been reported as a factor limiting natural attenuation processes such as biodegradation and photodegradation [23].

In this study, the concentrations of *p,p′*-DDE in fish muscle samples (< LOQ-40 μg/kg ww) were comparable with those from other freshwater fish found worldwide, such as Three Gorges Reservoir in China (1.78–18.44 ng/g ww) [24] and River Chenab in Pakistan (4.5–138.9 μg/kg ww) [25]. Moreover, *p,p′*-DDE was found to be the most predominant DDT metabolite in tilapia fish muscle collected from Assiut city and Bahr Shebeen Canal, Egypt, respectively [26,9].

The observed differences in pesticide concentrations in freshwater fish from different studies might be explained by variations in cropping patterns, improper usage of pesticides and their registration status, the distance of agricultural fields from the sampling sites, climate, and the efficiency of the analytical methodologies [27].

3.1.2. Antibiotics

The presence of chloramphenicol (CAP) and four nitrofuran metabolites [i.e., 3-amino-2-oxazolidone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ), 1-aminohydantonin (AHD) and semicarbazide (SEM)] were determined in Nile tilapia muscle samples along the Rosetta branch. The marker residues of the aforementioned nitrofuran banned parent drugs were furazolidone (FZD), furaltadone (FTD), nitrofurantoin (NFT) and nitrofurazone (NFZ), respectively.

Results indicate that 15 out of the 72 analyzed fish samples were contaminated with antibiotic residues as shown in (Table 2). Nitrofurazone (ranging from 8.6 to 52 μg/kg) was the most frequently detected

Table 2
Spatiotemporal distribution of antibiotics (μg/kg ww) in Nile tilapia muscle samples collected from the Rosetta branch of the river Nile, Egypt.

Antibiotics		Summer 2018			Autumn 2018			Winter 2019			Spring 2019		
		El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala
Contaminated samples %		17 %	0.0 %	33 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	100 %	43 %
Chloramphenicol	Conc. range (μg/kg)	BQL[b]	BDL	BQL- 0.17	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Mean[a](μg/kg)	BQL	–	0.17	–	–	–	–	–	–	–	–	–
	Freq. %	17 %	–	33 %	–	–	–	–	–	–	–	–	–
Nitrofurantoin	Conc. range (μg/kg)	BDL[c]	BDL	1.2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.1 - 2
	Mean (μg/kg)	–	–	1.2	–	–	–	–	–	–	–	–	1.6
	Freq. %	–	–	17 %	–	–	–	–	–	–	–	–	43 %
Nitrofurazone	Conc. range (μg/kg)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	8.6 - 52	BDL
	Mean (μg/kg)	–	–	–	–	–	–	–	–	–	–	26.137	–
	Freq. %	–	–	–	–	–	–	–	–	–	–	100 %	–

[a] The mean concentration of each season or sampling site represents the mean value of three months.
[b] BQL: Below quantification limit.
[c] BDL: Below detection limit.

antibiotic, followed by nitrofurantoin (ranging from 1.1 to 2 μg/kg) and chloramphenicol (ranging from < LOQ to 0.17 μg/kg). These were found in 12, 6 and 5% of the samples, respectively.

With respect to their spatial distribution, the results indicate that fish muscle samples from Sabal contained the highest mean concentrations of the antibiotics (26.14 μg/kg), followed by Tala (1.67 μg/kg) and El-Rahawy < LOQ). Fish samples collected from Sabal during spring and El-Rahawy during summer were contaminated with nitrofurazone and chloramphenicol residues, respectively. On the other hand, Tala samples were contaminated with nitrofurantoin (during summer and spring) and chloramphenicol during summer.

With regards to seasonal variations, samples collected during spring were the most contaminated with antibiotic residues (27.74 μg/kg), whereas summer samples had the lowest amounts of antibiotics (1.37 μg/kg). In contrast, no antibiotic residues were detected in the autumn and winter seasons.

Nitrofurans are broad-spectrum antibiotics which are commonly employed as feed additives for growth promotion, and mainly used for livestock, poultry, aquaculture and bee colonies for the prophylactic and therapeutic treatment of gastrointestinal and dermatological infections [28]. However, the use of nitrofurans for livestock production was completely banned in Europe and other countries due to their carcinogenic and genotoxic effects in humans [29]. Nitrofurans including FZD, NFT, NFZ, and FTD are rapidly metabolized following administration to AOZ, AHD, SEM, and AMOZ, respectively [30]. Nowadays, CAP is banned in the animal food-production chain as a veterinary antibiotic and is hardly used as human medication [31].

The results obtained demonstrated a continuation of the banned nitrofuran metabolites (AHD and SEM) and CAP antibiotics usage in food-producing species which may be due to their broad-spectrum, low cost, availability, and efficacy [32]. Among 102 analyzed imported seafood products, one swai fish sample tested positive for the SEM nitrofuran metabolite at the 2.5 ng/g level, while another shrimp sample was contaminated with CAP at 0.44 ng/g [33].

Table 3
Target hazard quotient (THQ) for detected pesticide residues in the muscles of Nile tilapia.

Season	Sampling site	Detected Pesticide	Mean Conc. (mg/kg)	ADI (mg/kg bw/d)	RfD (mg/kg bw/d)	THQ
Summer 2018	El-Rahawy	Chlorpyrifos	0.01	0.001	0.001	0.00636
	El-Rahawy	*p,p′*-DDE	BQL[a]	0.01	0.0003	–
	Sabal	Chlorpyrifos	0.03	0.001	0.001	0.01907
	Sabal	*p,p′*-DDE	0.01	0.01	0.0003	0.02119
	Tala	Chlorpyrifos	0.03	0.001	0.001	0.01907
	Tala	*o, p′*- DDT	0.01	0.01	0.0005	0.01271
	Tala	*p,p′*-DDE	0.03	0.01	0.0003	0.06357
Autumn 2018	El-Rahawy	Chlorpyrifos	0.0125	0.001	0.001	0.00795
	Sabal	Chlorpyrifos	0.05	0.001	0.001	0.03178
	Sabal	*p,p′*-DDE	BQL	0.01	0.0003	–
	Tala	Chlorpyrifos	0.03	0.001	0.001	0.01907
Winter 2019	El-Rahawy	Chlorpyrifos	0.015	0.001	0.001	0.00954
	El-Rahawy	*p,p′*-DDE	0.01	0.01	0.0003	0.02119
	Sabal	Chlorpyrifos	0.02	0.001	0.001	0.01271
	Sabal	Fludioxonil	0.03	0.37	Na[b]	0.00005
	Sabal	*p,p′*-DDE	0.012	0.01	0.0003	0.02543
	Sabal	Propiconazole	0.02	0.04	0.1	0.00013
	Sabal	Tebufenpyrad	0.016	0.01	0.02	0.00051
	Tala	Chlorpyrifos	0.03	0.001	0.001	0.01907
Spring 2019	El-Rahawy	Chlorpyrifos	0.01	0.001	0.001	0.00636
	Sabal	Atrazine	0.01	0.02	0.035	0.00018
	Sabal	Chlorpyrifos	0.026	0.001	0.001	0.01653
	Sabal	*p,p′*-DDE	0.012	0.01	0.0003	0.02543
	Tala	Chlorpyrifos	0.013	0.001	0.001	0.00826
	Tala	Fludioxonil	0.01	0.37	na	0.00002
	Tala	*p,p′*-DDE	0.01	0.01	0.0003	0.02119

[a] BQL:Below quantification limit.
[b] Na:not available ADI: Acceptable daily intake RfD: Reference dose.

Table 4
Target hazard quotient (THQ) for detected antibiotic residues in the muscles of Nile tilapia.

Antibiotics		RfD (mg/kg bw/day)	Summer 2018			Autumn 2018			Winter 2019			Spring 2019		
			El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala	El-Rahawy	Sabal	Tala
Chloramphenicol	Mean conc. (µg/kg)	0.0003	BQL[a]	BDL[b]	0.17	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	THQ	–	–	–	0.00036	–	–	–	–	–	–	–	–	–
Nitrofurantoin	Mean conc. (µg/kg)	0.07	BDL	BDL	1.2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.6
	THQ	–	–	–	0.00001	–	–	–	–	–	–	–	–	0.00001
Nitrofurazone	Mean conc. (µg/kg)	0.001	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	26.137	BDL
	THQ	–	–	–	–	–	–	–	–	–	–	–	0.01661	–

[a] BQL: Below quantification limit.
[b] BDL: Below detection limit RfD: Reference dose.

3.2. Health risk from consuming contaminated fish

The results of the target hazard quotient (THQ) values for individual pesticides and antibiotics from the consumption of contaminated Nile tilapia by the general population are presented in Tables 3 and 4, respectively.

The target hazard quotient (THQ) for each detected pesticide in fish samples were all far below the threshold value of 1. This implies that the exposure level is smaller than the RfD, suggesting that there is no significant risk associated with the consumption of fish contaminated with the detected pesticides from the studied sites. The highest values of THQ were observed for *p,p′*-DDE followed by chlorpyrifos representing 0.06 and 0.03, respectively. These findings agree with those reported from Tanta and Ismailia cities, Egypt [34]. On the contrary, Tongo and Ezemonye [35] showed that hazard quotient (HQ) and hazard index (HI) values for organochlorines pesticides exceeding 1, indicating the possibility of non-carcinogenic health risks to consumers especially children from consumption of cattle meat from the selected abattoirs located in Benin City, Southern Nigeria.

Calculated THQs for all detected antibiotics were much lower than the threshold value of 1, indicating that the consumption of Nile tilapia from the study sites is unlikely to cause any detrimental effects to consumers. THQ values for nitrofurazone, chloramphenicol, and nitrofurantoin were 0.01661, 0.00036 and 0.00001, respectively. These results are in coincidence with those obtained by Oyedeji et al. [36] who found that the presence of antibiotics residues in imported frozen poultry into Nigeria does not pose any immediate health risk.

In general, further studies are still needed to see whether synergistic, additive or antagonistic effects may produce on consumer health from exposure to multiple contaminants.

4. Conclusions

This study investigated the spatiotemporal variations of 100 pesticides and 5 antibiotics in Nile tilapia collected at monthly intervals over one year from 3 sampling sites along the Rosetta branch of the Nile, as well as the association between the health risks of detected contaminants and fish consumption in the Egyptian population. The findings of this study point out the close relationship that exists between the detected pesticides and their log K_{ow} since all the detected pesticides in Nile tilapia muscle, with the exception of atrazine, have **log** $K_{ow} > 3.0$ which means that they have a high bioaccumulation potential. In addition, the presence of pesticides and antibiotics residues in fish samples along the Rosetta branch means that the existing laws should be enforced, effective strategies for the treatment of the wastewaters discharged into the river Nile must be implemented and regular monitoring must be undertaken. Furthermore, this study confirmed that consumption of Nile tilapia from the Rosetta branch did not cause a significant potential health risk and can thus be considered to be safe for ordinary consumers.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.03.004.

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