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Assessment of trace element occurrence in Nile Tilapia from the Rosetta branch of the River Nile, Egypt: Implications for human health risk via lifetime consumption

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ABSTRACT

River pollution can harm human health through direct contact, drinking water, and the consumption of contaminated fish and irrigated agricultural products. Surface water and Nile tilapia (*Oreochromis niloticus*) samples were collected monthly from July 2022 to June 2023 at three sites (El-Rahawy, Sabal, and Tala) along the Rosetta Nile branch in Egypt to monitor the presence of eight trace elements. The potential human health risks from consuming contaminated fish were also assessed. Iron and manganese were consistently detected in all water samples across most seasons and locations, with concentrations generally below the WHO permissible levels. All 72 analyzed fish muscle samples were found to contain trace elements. The mean concentrations of metals in the fish muscle samples, in descending order, were: iron > zinc > copper > manganese > tin > antimony > lead > mercury. Significant spatial and seasonal variations were observed in both water and fish samples. El-Rahawy was identified as the most contaminated site, with summer exhibiting the highest contamination rate compared to other seasons. Fish samples collected from El-Rahawy demonstrated the highest bioconcentration factor (BCF) values for most elements, particularly mercury, lead, iron, manganese, and antimony. Target hazard quotient (THQ) calculations for the trace elements in Nile tilapia muscles revealed that all trace elements, except antimony, had THQ values below 1, suggesting that consuming Nile tilapia from these sites is unlikely to cause adverse health effects. However, THQ values for antimony exceeded the threshold of 1, indicating a potential health risk for consumers. Although the detected trace elements in the fish were below the permissible toxicity limits, some could pose a future threat to human health, necessitating further studies, ongoing monitoring, and preventive measures.

1. Introduction

The Nile River traverses through 11 African nations, among them Egypt, before it ultimately empties into the Mediterranean Sea. As it approaches Cairo, the river divides into two primary branches known as the Rosetta and Damietta, collectively forming the Nile Delta. This region has witnessed a surge in water demands over time due to factors such as population growth, urban expansion, industrial development, and agricultural activities (Mostafa et al., 2015). Regrettably, the Rosetta branch faces a significant challenge in the form of substantial daily inputs of contaminated water from diverse sources. These sources encompass industrial discharges, agricultural run-off, municipal wastewater, and the waste generated by fish breeding facilities. These collective inputs have detrimental repercussions on the aquatic ecosystem (Abbassy, 2018).

The Nile River serves as Egypt's primary source of freshwater. While

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numerous studies have examined pesticide and antibiotic residues in the Nile River (Eissa et al., 2022, 2021, 2020), research on other pollutants, such as heavy metals, has been somewhat limited in scope. Often, these investigations concentrate on a narrow selection of specific trace elements and employ relatively infrequent sampling frequencies (Talab et al., 2016). The presence of heavy metals in water bodies, such as the Nile River, poses a dual threat – not only to human health through water and food contamination but also to aquatic organisms themselves. Among these organisms, fish are particularly vulnerable as they come into contact with pollutants in the aquatic environment through various routes, including their skin, gills, and diet (Clasen et al., 2018). This makes fish consumption a significant source of human exposure to a wide range of environmental contaminants (Storelli, 2008). Consequently, the contamination of fish is a critical concern for human health. Water pollution emerges as a primary factor affecting the quality of fish production in their natural habitats, as pollutants find their way into the tissues of aquatic organisms and accumulate over time (Jiries et al., 2002). Fish represent a crucial protein source, and the continuously growing population significantly drives the demand for fish. Tilapia (*Oreochromis niloticus*) holds a prominent place in the Nile River and its tributaries, being the most widely distributed freshwater fish species in the region. Egypt, in particular, relies heavily on the production and consumption of tilapia (Talab et al., 2016).

Egypt is one of the leading producers of tilapia in the world. The production of tilapia fish from the Nile River in Egypt has been consistently high due to the favorable environmental conditions, including the presence of the River Nile and its tributaries, which provide suitable habitats for tilapia farming. Egypt's tilapia production was estimated to be over 1 million metric tons annually (Henriksson et al., 2017), representing about 77 % of the total fish production in Egypt (FAO, 2016). Since the 1990s, the cultivation of tilapia has been on a consistent growth trajectory, with an annual expansion rate exceeding 16 % over the last decade. Consequently, Egypt's farmed tilapia production has shown substantial growth in recent years, rising from 958,310 metric tons (MT) in 2018 to 1,079,748 MT by 2020 (FAO, 2022). Egypt's tilapia production accounted for approximately 71 % of the total African tilapia production and positioned it as the third largest tilapia producer globally, following China and Indonesia (Rossignoli et al., 2023). Nile tilapia is cultivated through both semi-intensive methods in earthen ponds and intensive techniques involving cages, ponds, concrete tanks, and integrated systems with land-based crops. The intensive culture of Nile tilapia in floating cages is another prevalent approach. These fish are stocked in floating cages at densities ranging from 60 to 100 fish/m^3, yielding between 25 and 40 kg/m^3 (El-Sayed, 2017).

Continuous monitoring of pollutants within the Nile River is a crucial endeavor, as it serves several vital purposes, including i) generating precise and quantitative data about the prevalence of pollutants in the river, which is invaluable for assessing their concentrations, ii) discerning the sources of these pollutants and tracking their environmental destiny, thus shedding light on their origins and fate, iii) ensuring adherence to environmental regulations and guidelines, helping to determine if pollutant levels meet established standards, iv) assessing the potential ramifications of these pollutants on both human health and the environment, and v) facilitating the refinement and optimization of advanced treatment technologies by providing insights into the specific pollutants that need to be addressed most urgently (Eissa et al., 2020).

In this context, the objectives of the scientific study are as follows: i) to systematically monitor and analyze the presence and concentration of 8 specific trace elements in surface water and Nile tilapia samples collected from various locations along the Rosetta branch of the River Nile in Egypt and their bioconcentration factors, ii) to calculate the target hazard quotient (THQ) for each trace element, providing a quantitative assessment of potential non-carcinogenic health risks for the local population consuming contaminated Nile tilapia over a lifetime, and ii) to communicate the study findings and their implications to relevant stakeholders, including local communities, policymakers, and environmental authorities, in order to raise awareness and inform decision-making processes related to fish consumption and water quality management along the Rosetta branch of the River Nile.

2. Materials and methods

2.1. Sampling and study location

The sampling approach was formulated based on the proximity of three drainage systems (El-Rahawy, Sabal, and Tala) that receive varying degrees of treated wastewater from wastewater treatment plants (WWTPs) before ultimately discharging their effluents into the Rosetta branch of the Nile River. Over a span of 12 sampling periods, spanning from July 2022 to June 2023, a total of seventy-two surface water and seventy-two Nile tilapia samples (*Oreochromis niloticus*) were collected in duplicate from three distinct sampling locations along the Rosetta branch approximately 1 km downstream from the discharge point of i) El-Rahawy drain (located in the Giza governorate; coordinates: 30°12'25.41" N and 31° 1′48.35" E), ii) Sabal drain (situated in the Minoufiya governorate; coordinates: 30°31'57.94" N and 30°50'53.20" E), and iii) Tala drain (located in Kafr El-Zayat, Gharbiya governorate; coordinates: 30°48'58.19" N and 30°48'37.60" E). This sampling approach aligns with the methodology outlined in our previously published study (Eissa et al., 2020) and is visually represented in Fig. 1.

Sampling was conducted on a monthly basis across all sites, with the aim of assessing the presence of 8 trace elements, i.e., mercury, lead, copper, zinc, iron, manganese, tin, and antimony. Detailed information about these selected trace elements, including their linearity, uncertainty, recovery percentages, limit of quantification (LOQ), and relative standard deviation (RSD), can be found in Table 1.

Surface water samples collected in plastic bottles were acidified with HNO_3 before being filtered through Whatman glass fiber filter paper and stored at 4°C until analysis. Fish samples, each weighing approximately two kilograms, were collected from each site with the assistance of local fishermen who accompanied the research team during the sampling events. For each sampling event, a total of eight Nile tilapia were collected from each site. The sampled fish were of commercial size, ranging from 200 to 250 g in weight, and were promptly transported in an ice box to the Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, located in Dokki, Giza, Egypt. Upon arrival, the fish samples underwent a thorough washing with tap water to eliminate any potential contaminants adhering to them. Afterwards, edible muscle tissue samples were obtained from the dorsal region of each fish. Muscle, being the primary edible portion frequently utilized in human health risk assessments, was homogenized and then stored at −20 °C until analysis.

2.2. Samples digestion and trace elements determination

Fish muscle samples were subjected to digestion following the method described by (Sepe et al., 2003). In a nutshell, one gram of each fish sample was carefully weighed into a microwave digestion vessel. Subsequently, 8 mL of concentrated nitric acid (HNO_3; Merck, Darmstadt, Germany) was introduced into the digestion vessel, followed by gentle shaking. Then, 2 mL of hydrogen peroxide (H_2O_2; Merck, Darmstadt, Germany) was added, and the mixture underwent digestion in the Milestone High-Pressure Microwave unit, specifically the Ethos Up model (Sorisole, Italy). The resulting solution, post-digestion, was transferred into a 50 mL volumetric flask. To this, 0.2 mL from the intermediate standard solution (100 mg/L), serving as an internal standard for lutetium (Lu), was added. The flask was then filled to the marked volume with deionized water. These digested samples were subsequently subjected to trace elements analysis using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Perkin-Elmer, Optima 8300, Waltham, United States). The quantified trace elements

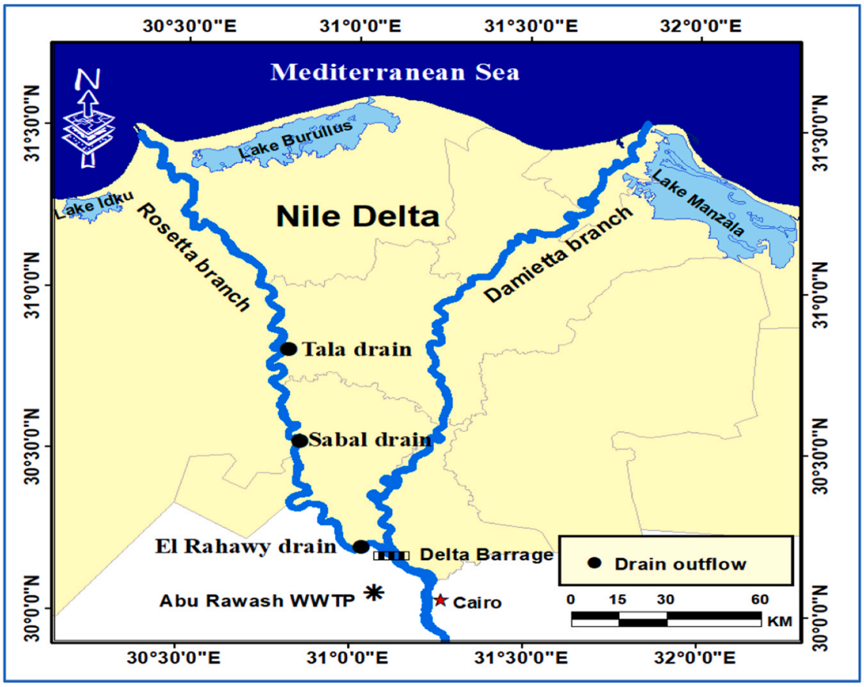

Fig. 1. The sampling sites of Nile tilapia along the Rosetta branch, river Nile, Egypt.

Table 1
Recovery rates, relative standard deviations (RSD), linearity, uncertainty, and limits of quantification (LOQ) of selected trace elements.

Element	Wavelength (nm)	LOQ (μg/L)	Recovery %	Linearity	RSD %	Expanded uncertainty %
Mercury	194.168	0.03	93.216	0.9999	5.15	23.67
Lead	220.353	0.05	99.523	0.9994	5.39	22.85
Copper	327.393	1	108.108	0.9999	8.76	27.30
Zinc	202.548	1	107.593	0.9993	6.15	23.91
Iron	238.204	1	102.410	0.9995	7.30	25.26
Manganese	257.61	1	103.098	0.9993	4.22	21.94
Tin	189.927	1	107.680	0.9996	4.16	21.91
Antimony	206.836	0.4	101.547	0.9990	4.88	23.57

by ICP-OES were Hg, Pb, Cu, Zn, Fe, Mn, Sn, and Sb, and their respective wavelengths (in nm) were 194.168, 220.353, 327.393, 202.55, 238.204, 257.61, 189.927, and 206.836, respectively.

2.3. Bioconcentration factor

The bioconcentration factor (BCF) was determined by calculating the ratio of the concentration of trace elements in fish to the concentration of trace elements in the surrounding water under conditions of equilibrium, as previously described by (Zhelyazkov et al., 2018). The BCF estimation was based on the following assumptions: a) The environmental conditions, fish feeding, excretion, and chemical concentrations in the ambient water had reached a state of equilibrium; b) The kinetic BCF was considered equivalent to the equilibrium BCF; and c) The BCF was calculated as the ratio of the steady-state trace element concentration in fish to that in water, following first-order kinetics (Orata and Birgen, 2016; Rahayu et al., 2020). The BCF was represented mathematically as follows:

$$BCF = \frac{Concentration\ of\ trace\ element\ in\ fish\ muscel(mg/kg\ FW)}{Concentration\ of\ trace\ element\ in\ water(mg/L)}$$

2.4. Human risk assessment

To assess the potential non-carcinogenic health risks associated with extended exposure to various trace elements via Nile tilapia consumption, the following human health risk indices were determined:

a) $EDI = \frac{C \times FIR}{BW}$ (US EPA, 2013)
   where, EDI = estimated daily intake of each trace element (μg/kg BW/day); C = concentration of trace element Nile tilapia (mg/kg FW); FIR = fish ingestion rate (38.14 g/person/day; FAO, 2013); BW = body weight (60 kg/person).

b) $THQ = \frac{EDI \times ED \times EF}{AT \times RfD} \times 10^{-3}$ (Ahmed et al., 2016)
   where, THQ = target hazard quotient; ED = exposure duration (70 years, which is equivalent to the average human lifetime); EF = exposure frequency (365 days per year); AT = the averaging time for non-carcinogenic risks (365 day/year × ED); RfD = oral reference dose of Hg, Pb, Cu, Zn, Fe, Mn, Sn, and Sb are 0.0003, 0.004, 0.04, 0.3, 0.7, 0.14, 0.6, and 0.0004 mg/kg/day, respectively (US EPA, 2013).

c) $HI = \sum_{i=k}^{n} THQs$ (Haque et al., 2021)

   where, HI = hazard index.

d) $CR = \frac{EDI \times ED \times EF \times CSf}{AT} \times 10^{-3}$ (Vieira et al., 2011)
   where, CR = carcinogenic risk; CS*f* = oral slope factor of Hg, Pb, Cu, Zn, Fe, Mn, Sn, and Sb are 0.0005, 0.0000085, 0.0885, 0.38, 0.01, 0.14, 0.0005, and 0.0005 mg/kg/day, respectively, (USEPA, 2019).

2.5. Quality assurance

Quality assurance measures were implemented throughout the laboratory procedures, aligning with ISO/IEC 17025:2017 standards. Additionally, these methods and instruments underwent a rigorous validation process, which included audits and accreditation by the Finnish Accreditation Service (FINAS), located in Helsinki, Finland. To assess the effectiveness of the analysis, blank and spiked samples were subjected to the same method described above. The correlation coefficients ranged between 0.9990 and 0.9999, indicating excellent linearity for all elements. The average recoveries for the 8 selected trace elements in fish samples ranged from 93.22 % to 108.11 %. Repeatability, as indicated by the relative standard deviation (RSD), remained below 9 % for all selected elements. The expanded uncertainty values were relatively consistent across all elements, ranging from 21.91 % to 27.30 %. The limits of quantification (LOQ) for trace elements in fish samples ranged from 0.03 to 1 mg/kg, as detailed in Table 1.

3. Results and discussion

3.1. Spatiotemporal variations of trace elements in water samples collected from the Rosetta Nile branch

Results of trace elements presented in Table 2 revealed spatiotemporal variations of their concentrations in water sampled from the Rosetta Nile branch at three different locations, i.e., El-Rahawy, Sabal, and Tala. Overall, El-Rahawy site recorded the highest frequency of trace elements detection, followed by Tala site while Sabal site displayed the least frequency. The highest frequency of the trace elements corresponded to summer season, followed by autumn and winter, whereas spring season exhibited the least frequency. Among the detected trace elements, iron and manganese were detected in all samples. Iron consistently shows the highest concentrations among all elements analyzed, exceeding the WHO permissible limit of 0.30 mg/L at El-Rahawy during summer 2022 (0.339 mg/L) and winter 2023 (0.498 mg/L). High iron levels could be attributed to both natural sources like geological formations and anthropogenic activities such as agricultural runoff and industrial discharges. Antimony concentrations were highest at El-Rahawy during summer 2022 (0.124 mg/L), significantly exceeding the WHO permissible limit of 0.02 mg/L, while its levels at other drains and seasons were much lower and within the permissible limits. In the El-Rahawy and Tala sites, zinc was detected in all seasons except spring, while in the Sabal site it was only measured in spring. Lead was detected only twice in summer and winter at El-Rahawy and Tala sites, respectively. The highest concentration of mercury (0.007 mg/L) was observed at the Sabal drain during winter 2023, exceeding the WHO permissible limit of 0.006 mg/L, while its levels at El-Rahawy and Tala drains were generally lower and within the permissible limits. According to (WHO, 2011), the permissible concentrations of mercury, lead, copper, zinc, iron, manganese, and antimony in freshwater are 0.006 mg/L, 0.01 mg/L, 2.0 mg/L, 3.0 mg/L, 0.3 mg/L, 0.4 mg/L, and 0.02 mg/L, respectively. In the current study, mercury, iron, and antimony levels occasionally exceeded the WHO permissible limits at certain sites and seasons, while the concentrations of other elements were generally within the acceptable range as shown in Table 2. (Ghannam, 2021) conducted an investigation on the concentrations of lead, copper, zinc, iron, and manganese in the River Nile across three locations — El-Tbeen district, Manyal district, and El-Kanater El-Kairia city — from autumn 2018 to summer 2019. The metal concentrations varied considerably depending on the sampling site. Iron, lead, and manganese often surpassed acceptable levels, posing potential health risks. Specifically, lead ranged from 0.009 to 0.156 mg/L, copper from 0.002 to 0.213 mg/L, zinc from 0.015 to 0.526 mg/L, iron from 0.12 to 7.29 mg/L, and manganese from 0.01 to 1.04 mg/L. These findings are consistent with our study, showing considerable variability in metal concentrations depending on the sampling site and time. The order of metal concentrations was iron > manganese > zinc > copper > lead, aligning with our results. The increased concentrations of trace elements in the River Nile can be attributed to a combination of factors, including lower water levels during winter and spring (Elewa, 2010), decomposition of dead aquatic plants (Harguinteguy et al., 2016), high evaporation rates during periods of low precipitation (El-Sheekh, 2016), and the release of metals from sediments due to changes in pH, redox conditions, or microbial activity (Abdel-Satar et al., 2017).

3.2. Spatial and temporal distribution of trace elements in Nile tilapia along the Rosetta Nile branch

The levels of the eight trace elements in the muscles of Nile tilapia significantly varied according to the sampling time and location. Among these elements, copper, zinc, iron, manganese, and tin reached their

Table 2
Spatiotemporal distribution of trace elements (mg/L) in surface water samples collected from the Rosetta Nile branch, Egypt, at the mouth of three agricultural drains (i.e., El-Rahawy, Sabal, and Tala) during the four seasons.

Element	El-Rahawy				Sabal				Tala			
	Summer 2022	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*
Mercury (0.006)[¥]	0.0006 ± 0.0001	ND[†]	0.0010 ± 0.00007	<LOQ	<LOQ	<LOQ	0.0072 ± 0.0014	ND	0.0023 ± 0.0013	<LOQ	ND	0.0016 ± 0.0008
Lead (0.01)	0.0031 ± 0.0002	<LOQ	<LOQ	ND	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ	0.0086 ± 0.0041	ND
Copper (2.0)	<LOQ[‡]	0.0943 ± 0.0342	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0501 ± 0.0045	0.1521 ± 0.0228	<LOQ	<LOQ
Zinc (3.0)	0.0684 ± 0.0095	0.0653 ± 0.0073	0.0543 ± 0.0038	<LOQ	<LOQ	<LOQ	<LOQ	0.0546 ± 0.0043	0.0582 ± 0.0029	0.0914 ± 0.0073	0.0779 ± 0.0299	<LOQ
Iron (0.30)	0.3396 ± 0.2135	0.2564 ± 0.1805	0.4982 ± 0.1574	0.0692 ± 0.0357	0.2066 ± 0.2139	0.1235 ± 0.0478	0.0997 ± 0.0256	0.2970 ± 0.4913	0.1512 ± 0.1286	0.2020 ± 0.2242	0.0829 ± 0.0229	0.0893 ± 0.0066
Manganese (0.40)	0.1169 ± 0.0574	0.1120 ± 0.0247	0.0889 ± 0.0226	0.0546 ± 0.0032	0.0911 ± 0.0487	0.1069 ± 0.0349	0.1049 ± 0.0031	0.0620 ± 0.0065	0.0607 ± 0.0174	0.1243 ± 0.0402	0.0956 ± 0.0260	0.0837 ± 0.0040
Tin (-)[Φ]	0.0681 ± 0.0063	0.0559 ± 0.0040	0.0610 ± 0.0099	<LOQ	0.0564 ± 0.0073	<LOQ	<LOQ	0.1609 ± 0.0097	<LOQ	0.0643 ± 0.0051	<LOQ	<LOQ
Antimony (0.02)	0.1236 ± 0.1127	0.0014 ± 0.0002	0.0019 ± 0.0006	<LOQ	0.0022 ± 0.0008	0.0019 ± 0.0008	0.0026 ± 0.0016	<LOQ	0.0024 ± 0.0011	0.0025 ± 0.0007	0.0025 ± 0.0023	<LOQ

[¥] Permissible concentrations (mg/L) of trace elements according to WHO (2011).
[Φ] Not provided.
[†] Not detected.
[‡] Below limit of quantification.

highest mean concentrations, measuring 22.98, 25.65, 43.78, 13.30, and 2.65 mg/kg, respectively, in Nile tilapia caught during the spring. Samples collected near the discharge point of the El-Rahawy drain showed the highest mean concentrations (mg/kg) of lead (0.27), copper (22.98), zinc (25.65), iron (43.78), manganese (13.30), and antimony (1.06), irrespective of the sampling time (Table 3). Nile tilapia samples collected near Sabal drain exhibited the highest mean concentration of mercury (0.11 mg/kg) during winter and tin (2.65 mg/kg) during spring. The descending order of trace elements mean concentrations in Nile tilapia muscles, regardless of the sampling time and site, can be well-described as follows: iron > zinc > copper > manganese > tin > antimony > lead > mercury. Nevertheless, in one sample, copper, zinc, and antimony surpassed the maximum permissible limits (MPLs) established by FAO/WHO for the analyzed trace elements, while manganese exceeded the MPLs in four Nile tilapia samples. Approximately 71 % of the samples that violated the permissible levels were collected near the El-Rahawy drain, with the remaining percentage originating from the samples gathered near the Tala drain (Table 3). This emphasizes the need for immediate action to mitigate pollution sources and ensure the sustainability of the aquatic ecosystem and the health of local communities relying on fish as a food source.

In harmony with the present findings, (Talab et al., 2016) observed irregular distributions of heavy metal accumulation in tilapia fish muscles collected from the main irrigation canals of the Nile Delta, Egypt, spanning from spring 2014 to winter 2015. The descending order of accumulation was reported as follows (mg/kg dry weight): iron (20.00) > zinc (7.18) > manganese (2.41) > copper (1.45) > lead (1.60) > cadmium (0.17), and only zinc and lead showed higher values than our results. Similarly, in the muscles of tilapia fish collected from the Langat River, Malaysia, (Taweel et al., 2013) noted that zinc exhibited the highest concentrations (20.85–26.13 μg/g dry weight), followed by nickel (3.69–3.86 μg/g dry weight) among the measured metals. Contrary to our findings, fish accounted for approximately 91.5 % of mercury notifications in the European Union Rapid Alert System for Food and Feed database (RASFF) from 2000 to 2022 (Eissa et al., 2023).

Fish have a propensity to accumulate metals in their tissues at levels significantly exceeding those of the surrounding environment. This accumulation occurs through processes such as absorption through gills or ingestion of contaminated food and sediment (Malik et al., 2010). The extensive utilization of trace elements in diverse domestic and industrial activities has led to the anthropogenic influx of these elements into water bodies (Kaushik et al., 2009).

In terms of spatial distribution, the data revealed that fish muscle samples obtained from El-Rahawy exhibited the highest mean concentrations of trace elements (58.6 mg/kg), followed by Tala (41.1 mg/kg) and Sabal (27.7 mg/kg). This indicates that the specific agricultural practices and industrial activities surrounding each drain likely contribute differently to the trace element profile of the water and consequently the fish. The Abu-Rawash Wastewater Treatment Plant (WWTP) receives approximately 1.45 million cubic meters of raw wastewater daily, surpassing its capacity of around 1.20 million cubic meters per day (Mostafa et al., 2015). Consequently, an excess of approximately 250,000 cubic meters of wastewater is discharged directly into the Barakat drain, then into the El-Rahawy drain, and ultimately into the Rosetta Branch of the Nile without prior treatment, thereby elevating the risk of water and fish contamination (Mostafa and Peters, 2016). The discharge of domestic wastewater could elevate concentrations of certain trace elements (such as Cu, Zn, Pb, and Fe) due to the frequent use of household products like cleaning agents, toothpaste, and cosmetics (Alloway, 2013).

The mean concentrations of trace elements in fish muscle samples were highest during spring (53.5 mg/kg), followed by summer (53.0 mg/kg), autumn (34.7 mg/kg), and winter (28.6 mg/kg). During autumn and spring, the highest mean concentrations of trace elements were observed in fish samples collected near the El-Rahawy drain, with values of 63.1 and 105.9 mg/kg, respectively. Conversely, fish samples collected near the Tala drain exhibited the highest mean concentrations of trace elements during summer (62.4 mg/kg) and winter (58.5 mg/kg) seasons. The seasonal variability in a specific site is likely influenced by the neighboring urban, industrial, and agricultural activities (Eissa et al., 2022).

Kafr El-Zayat city hosts several factories including those producing super-phosphate and sulfur compounds (El-Malia Company), as well as oil and soap industries (El-Malh and Soda Company), and a pesticides factory. These factories discharge their industrial effluents either directly or indirectly into the Rosetta branch at the Tala sampling site. Additionally, the elevated concentrations of trace elements during the spring and summer seasons may be attributed to surface runoff from nearby agricultural areas. Such runoff could potentially contribute to the release of metals such as chromium, zinc, copper, and cadmium, as these metals are commonly found in fertilizers or pesticides (Alloway, 2013).

3.3. Building up of trace elements in muscles of Nile tilapia

The bioconcentration factor (BCF) of the eight trace elements exhibited significant variability based on both sampling time and location (Table 4). Fish samples collected from El-Rahawy demonstrated the highest BCF values for mercury (116.7) and lead (87.1) during the summer season, for iron (632.6) and manganese (243.7) in winter, and for antimony (757.1) in autumn. At the Tala site, the highest BCF values were observed for copper (239.5) in summer and zinc (231.3) in winter. Tin exhibited its highest BCF value (16.5) at the Sabal site during spring. These BCF results affirm that El-Rahawy is the most contaminated site, followed by Tala, while Sabal demonstrates comparatively lower BCF values for all detected trace elements except tin. The findings also underscore the spatial and temporal fluctuations in trace element concentrations along the River Nile. The seasonal variations in BCF values may be influenced by factors such as water quality, agricultural practices, and the physiological state of the fish.

The term BCF is used to describe the capacity of a contaminant to accumulate in an organism's tissues from the environment. Bioconcentration happens when a substance is absorbed faster than it is eliminated, leading to its buildup in an animal's tissues (Arnot and Gobas, 2006). A bioconcentration factor (BCF) value of less than 1 indicates that the metal does not undergo biomagnification. In the present study, all tested trace elements exhibited a BCF greater than 1, except for manganese at the Sabal site during the summer season, which had a BCF of 0.7. This indicates a potential risk associated with the increased consumption of Nile tilapia, despite most elements in the freshwater being within permissible limits, except for mercury, iron, and antimony (Table 2). Contrarily, other studies reported different findings in various fish species. For instance, (Varol et al., 2017) found that in rainbow trout (*Oncorhynchus mykiss*) from the Karakaya Dam Reservoir, the BCF values for ten metals were below 1, with nickel having the highest BCF value of 0.821, followed by arsenic (0.655) and chromium (0.625). Similarly, (Kelly et al., 2008) demonstrated that the BCF values for 18 elements (excluding mercury) in farmed Atlantic (*Salmo salar*), coho (*Oncorhynchus kisutch*), and chinook salmon (*Oncorhynchus tshawytscha*) from Canada were all below 1, with cadmium showing the lowest BCF values.

3.4. Health risk from consuming contaminated fish

The findings detailing the estimated daily intake (EDI) of the eight trace elements, derived from the consumption of Nile tilapia muscles collected from three distinct locations along the River Nile across all four seasons, are summarized in Table 5. These EDI values correlate significantly with the sampling time and location. While the EDI values for the identified trace elements remained below the tolerable daily intake (TDI), the highest amount of a trace element that can be consumed daily for a lifetime with no risks to living organism's life, certain exceptions were noted. Notably, antimony exhibited a TDI/EDI ratio of 1.28,

Table 3
Spatiotemporal distribution of trace elements (mg/kg FW) in Nile tilapia muscle samples collected from the Rosetta Nile branch, Egypt, at the mouth of three agricultural drains (i.e., El-Rahawy, Sabal, and Tala) during the four seasons.

Element (MPL[†], mg/kg)		**El-Rahawy**				**Sabal**				**Tala**			
		Summer 2022	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*
Mercury	Concentration range	<LOQ[a]–0.09	0.04–0.09	0.04–0.08	ND[‡]	<LOQ - 0.03	ND	<LOQ–0.16	<LOQ	<LOQ–0.12	ND	<LOQ–0.06	<LOQ–0.06
(0.50[a])	Mean**	0.07	0.07	0.06	-	0.03	-	0.11	<LOQ	0.08	-	0.05	0.06
	Frequency %	50	60	29	-	50	-	86	38	67	-	75	43
Lead	Concentration range	0.27	ND	<LOQ–0.12	0.14–0.15	0.11	ND	<LOQ	<LOQ	0.1–0.21	ND	<LOQ	ND
(0.30[a])	Mean	0.27	-*	0.12	0.15	0.11	-	<LOQ	<LOQ	0.15	-	<LOQ	-
	Frequency %	17	-	43	50	17	-	29	13	50	-	25	-
Copper	Concentration range	<LOQ–12.9	<LOQ–4.0	<LOQ–4.4	9.7–42.9	13.7–15.7	<LOQ	<LOQ–1.6	<LOQ–5.4	<LOQ–13.9	<LOQ	1.9–11.2	1.4–13.7
(30.0[b])	Mean	12.40	2.10	2.72	22.98	14.79	<LOQ	1.27	4.25	12.00	<LOQ	6.875	6.09
	Frequency %	83	80	100	100	50	67	86	100	67	33	100	100
Zinc	Concentration range	3.6–8.7	7.1–17.1	<LOQ–7.8	5.4–46	7.5–12.2	3.7–5.1	4.3–7.1	4.3–8.4	5.8–10.4	7.1–7.5	12.5–24.2	5.5–23.7
(40.0[b])	Mean	5.15	11.76	6.14	25.65	10.18	4.40	5.47	6.29	7.88	7.27	18.03	10.60
	Frequency %	100	100	100	100	100	100	100	100	100	100	100	86
Iron	Concentration range	3.3–64.7	5.1–76.3	<LOQ–5.3	5.7–91.5	7.2–48.8	13.2–39.5	3–11.5	3.7–24.4	9.4–87.6	5.7–6.5	21.2–36.4	1.5–14.4
(100[b])	Mean	31.78	32.36	3.58	43.78	19.31	22.07	7.09	12.75	39.53	6.03	28.50	8.04
	Frequency %	100	100	100	100	100	100	100	100	100	100	100	100
Manganese	Concentration range	<LOQ–2.5	<LOQ–16.2	<LOQ	<LOQ–17.7	<LOQ–0.6	<LOQ	<LOQ	<LOQ	<LOQ–4.3	<LOQ–1.3	3.4–6.1	<LOQ–5.7
(5.0[b])	Mean	2.50	15.75	<LOQ	13.30	0.06	<LOQ	<LOQ	<LOQ	2.75	1.30	4.88	3.92
	Frequency %	100	100	86	100	100	100	86	100	100	100	100	100
Tin	Concentration range	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ - 3.3	<LOQ	<LOQ	ND	<LOQ
(50.0[c])	Mean	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-	2.65	<LOQ	<LOQ	-	<LOQ
	Frequency %	50	80	57	50	33	33	-	75	17	67	-	86
Antimony	Concentration range	<LOQ	<LOQ–1.20	<LOQ–0.51	<LOQ–0.04	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ	0.42–0.59	ND
(1.0[d])	Mean	<LOQ	1.06	0.51	0.04	<LOQ	<LOQ	<LOQ	-	<LOQ	<LOQ	0.50	-
	Frequency %	33	80	100	75	50	67	43	-	67	100	100	-

[†] Maximum permissible limit
[‡]Not detected
[a]EC (2006),
[*] not calculated
[**] The mean concentration of each season or sampling site represents the mean value of three months.
[b] WHO (1993),
[c] FAO (1983),
[d] FAO/WHO (2002)
[a] Below limit of quantification.

Table 4
Bioconcentration factor (BCF) of eight trace elements in the muscles of Nile tilapia collected from the Rosetta branch of the Nile River at the mouth of three agricultural drains (i.e., El-Rahawy, Sabal, and Tala) during the four seasons.

Element	El-Rahawy				Sabal				Tala				Mean		
	Summer 2022	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*El-Rahawy*	*Sabal*	*Tala*
Mercury	116.7	-‡	57.7	-	-	-	15.5	-	35.4	-	-	37.5	87.2	15.5	36.4
Lead	87.1	-	-	-	-	-	-	-	-	-	-	-	87.1	-	-
Copper	-	22.3	-	-	-	-	-	-	239.5	-	-	-	22.3	-	239.5
Zinc	75.3	180.2	113.1	-	-	-	-	115.3	135.4	79.5	231.3	-	122.9	115.3	148.7
Iron	93.6	126.2	7.2	632.6	93.5	178.7	71.1	42.9	261.4	29.9	344.0	90.0	214.9	96.5	181.3
Manganese	21.4	140.6		243.7	0.7			-	45.3	10.5	51.0	46.8	135.2	0.7	38.4
Tin	-	-	-	-	-	-	-	16.5	-	-	-	-	-	16.5	-
Antimony	-	757.1	268.4	-	-	-	-	-	-	-	202.4	-	512.8	-	202.4

‡ No enough data for calculation

indicating potential health hazards for consumers, in the autumn season at the EL-Rahawy site. Additionally, mercury raised concerns due to its TDI/EDI ratio of 10, suggesting potential threats to consumer health, in the winter season at the Sabal site. The EDI is the daily amount of a contaminant consumed through food, water, or other sources, calculated to assess exposure levels and ensure they remain within safe limits established by regulatory agencies. The TDI represents the maximum quantity of a contaminant that can be ingested daily over a lifetime without significant health risks. According to the United States Environmental Protection Agency (USEPA, 2019), the TDI values for mercury, lead, copper, zinc, iron, manganese, tin, and antimony are 0.71, 1.5, 500, 300, 800, 140, 600, and 0.86 µg/kg body weight/day, respectively. Based on the EDI values presented in Table 5, all tested elements had EDIs below their respective TDI values, indicating that consuming Nile tilapia from the River Nile is safe. Similar findings were reported by (Varol et al., 2017), who found that the EDI values of ten trace elements in the muscles of rainbow trout were well below the TDI limits, particularly for nickel and lead, showing no health risks from consuming the examined fish. Likewise, (Adegbola et al., 2021) reported that the EDI values of cadmium, lead, and arsenic in the gills of *Clarias gariepinus* from the Ogun River were all below TDI limits.

Nile tilapia samples possessed target hazard quotient (THQ) below the reference value (1) for all the tested trace elements, regardless sampling time or location, except for the antimony, which revealed a THQ of 1.68 at El-Rahawy site during autumn season. Also, antimony had a THQ of 0.81 and 0.80 during winter season at the El-Rahawy and Tala sites. Results also revealed that mercury and copper exhibit possible toxicity as they recorded THQ of 0.237 and 0.365, respectively (Table 5). Overall, the descending order of THQ values for each trace element through Nile tilapia consumption was antimony > mercury > copper > manganese > lead > zinc > iron > tin. The THQ indicates the level of human health risk from exposure to a contaminant. THQ values greater than 1 suggest a higher probability of experiencing long-term non-carcinogenic effects, while values less than 1 indicate no significant health risks for the exposed population. If the THQ equals or exceeds 1, it signifies a need for intervention and protective measures due to the higher probability of adverse health effects. In the current study, the THQ values for eight trace elements across three sampling locations during four seasons were all below 1, except for antimony at the El-Rahawy site in autumn, which had a THQ of 1.684. These findings align with those reported for the main irrigated canals of the Nile Delta, Egypt (Talab et al., 2016). Similarly, (Varol et al., 2017) found that the THQ values for ten metals in rainbow trout were all below 1. Additionally, (Finley et al., 2012) reported THQ values below 1 for arsenic, cadmium, chromium, lead, nickel, and cobalt in *Clarias gariepinus* and *Sarotherodon melanotheron* collected from the Eleyele and Ogun rivers. The THQ values below 1 in the current study indicate no health risk for adults from ingesting trace elements in Nile tilapia.

The carcinogenic risk (CR) is a quantitative measure used to estimate the potential cancer risk associated with exposure to certain chemicals or substances such as trace elements. CR values below 10^{-6} signify negligible cancer risks, while those surpassing 10^{-4} indicate potential cancer hazards, rendering food items with CR values above 10^{-4} unacceptable. Foods falling within the range of CR values between 10^{-6} and 10^{-4} are generally deemed acceptable. In the present investigation, Nile tilapia samples gathered from the Sabal and Tala sites exhibited CR values within a non-toxic range, barring an exception observed for iron during the summer season at the Tala site, registering a CR of 0.126×10^{-4}. Conversely, several trace elements posed cancer risks at the El-Rahawy site, with CR values surpassing 10^{-4}. For instance, copper exhibited a CR of 1.24×10^{-4} during the spring season. Furthermore, zinc demonstrated CR values exceeding 10^{-4} across the summer, autumn, winter, and spring seasons, recording 12.44, 28.41, 14.83, and 61.96×10^{-4}, respectively. Similarly, iron and manganese showcased CR values surpassing the toxic threshold during the summer, autumn, and spring seasons (Table 5). The CR is a measure used in environmental

Table 5
Estimated daily intake (EDI; μg/kg BW/day), target hazard quotient (THQ), carcinogenic risk (CR), and total hazard index (HI) for trace elements in the muscles of Nile tilapia collected from the Rosetta branch of the Nile River, Egypt, at the mouth of three agricultural drains (i.e., El-Rahawy, Sabal, and Tala) during the four seasons.

Element		El-Rahawy				Sabal				Tala				HI (total)		
		Summer 2022	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*	*Summer 2022*	*Autumn 2022*	*Winter 2023*	*Spring 2023*	**El-Rahawy**	**Sabal**	**Tala**
Mercury	EDI	0.044	0.044	0.038	-¥	0.019	-	0.071	-	0.051	-	0.032	0.038	0.424	0.301	0.403
	THQ	0.148	0.148	0.127	-	0.064	-	0.237	-	0.170	-	0.106	0.127			
	CR ($\times10^{-8}$)	2.22	2.22	1.91	-	0.95	-	3.56	-	2.54	-	1.59	1.91			
Lead	EDI	0.172	-	0.076	0.092	0.070	-	-	-	0.095	-	-	-	0.085	0.017	0.024
	THQ	0.043	-	0.019	0.023	0.017	-	-	-	0.024	-	-	-			
	CR ($\times10^{-8}$)	0.15	-	0.065	0.078	3.5	-	-	-	4.77	-	-	-			
Copper	EDI	7.882	1.335	1.729	14.604	9.402	-	0.807	2.702	7.628	-	4.370	3.871	0.639	0.323	0.397
	THQ	0.197	0.033	0.043	0.365	0.235	-	0.020	0.068	0.191	-	0.109	0.097			
	CR ($\times10^{-8}$)	6700	1130	1470	12400	470	-	40.4	135	381	-	219	194			
Zinc	EDI	3.274	7.475	3.903	16.305	6.471	2.797	3.477	3.998	5.009	4.621	11.458	6.738	0.103	0.056	0.092
	THQ	0.011	0.025	0.013	0.054	0.022	0.009	0.012	0.013	0.017	0.015	0.038	0.022			
	CR ($\times10^{-8}$)	124400	284100	148300	619600	324	140	174	200	250	231	573	337			
Iron	EDI	20.201	20.570	2.276	27.826	12.275	14.029	4.507	8.105	25.128	3.833	18.117	5.111	0.101	0.056	0.074
	THQ	0.029	0.029	0.003	0.040	0.018	0.020	0.006	0.012	0.036	0.005	0.026	0.007			
	CR ($\times10^{-8}$)	2020	2060	228	2780	614	701	225	405	1260	192	906	256			
Manganese	EDI	1.589	10.012	-	8.454	0.038	-	-	-	1.748	0.826	3.099	2.492	0.143	0.0003	0.058
	THQ	0.011	0.072	-	0.060	0.003	-	-	-	0.012	0.006	0.022	0.018			
	CR ($\times10^{-8}$)	22200	140200		118400	1.91	-	-	-	87.4	41.3	155	125			
Tin	EDI	-	-	-	-	-	-	-	1.685	-	-	-	-	-	0.0028	-
	THQ	-	-	-	-	-	-	-	0.003	-	-	-	-			
	CR ($\times10^{-8}$)	-	-	-	-	-	-	-	84.2	-	-	-				
Antimony	EDI	-	0.674	0.324	0.025	-	-	-	-	-	-	0.319	-	2.559	-	0.798
	THQ	-	1.684	0.810	0.064	-	-	-	-	-	-	0.800	-			
	CR ($\times10^{-8}$)	-	33.7	16.2	1.27	-	-	-	-	-	-	16	-			
HI (total)		0.68308	1.99203	1.01616	0.86577	0.61218	0.02936	0.57217	0.32833	0.46502	0.07764	1.33225	0.48336	4.557	1.539	2.358

† Oral reference dose
‡ below limit of quantification.
¥ No enough data for calculation

health and risk assessment to estimate the probability of an individual developing cancer over a lifetime due to exposure to carcinogenic agents such as trace elements (Zhong et al., 2018). Carcinogenic risks higher than 10^{-4} are likely to increase the likelihood of cancer (Yin et al., 2015). In the present study, fish samples collected near the El-Rahawy drain showed higher cancer risks for consumers compared to those from the other two locations, Sabal and Tala. The CR values for eight trace elements across the four seasons at the Sabal and Tala sites were below the toxic level (CR $< 10^{-4}$). However, at the El-Rahawy site, the CR values for zinc exceeded 10^{-4} during all four seasons, and iron and manganese had CR values above 10^{-4} throughout the year except in winter. Copper showed only a higher CR value ($>10^{-4}$) in the spring season (Table 5). Additionally, CR values for *Clarias gariepinus* and *Sarotherodon melanotheron* from the Eleyele and Ogun Rivers exceeded the non-carcinogenic limit (10^{-4}) for arsenic, cadmium, and nickel, while chromium and lead were below 10^{-4} (Finley et al., 2012). Moreover, the CR value of arsenic in the muscles of rainbow trout did not exceed the carcinogenic level, recording a value of 10^{-6} (Varol et al., 2017).

The total hazard index (HI) results, calculated for all trace elements in one season at a sampling site, revealed that samples from the El-Rahawy site in autumn and winter, and from the Tala site in winter, had HI values greater than 1. However, these values are well below the reference value of 10, indicating no health risk for adults in Egypt from ingesting eight trace metals found in Nile tilapia (Table 5). The total HI for a single trace element over the entire year at one sampling site showed that only antimony at the El-Rahawy site exceeded 1, with a value of 2.559. Despite this, the value is significantly below the toxic threshold of 10, posing no health risk to consumers. Although all HI values are below toxic levels, the El-Rahawy site, particularly in autumn, may present potential risks in the future if preventive measures are not implemented. In general, further studies are still needed to see whether synergistic, additive or antagonistic effects may produce on the consumer health from exposure to multiple contaminants. Moreover, Fe accumulates in water due to its mobilization and release from Fe-rich-soil sources (Mora et al., 2009).

4. Conclusions

This study investigated the spatiotemporal variations of eight trace elements in water and Nile tilapia from the Rosetta Nile branch and assessed the associated health risks. The trace element concentrations exhibited spatial and temporal variability, influenced by factors such as seasonal changes, agricultural runoff, and industrial discharges. Iron was the most abundant element in fish muscle, followed by zinc, copper, manganese, tin, antimony, lead, and mercury. The presence of trace elements in fish highlights the need for enforcing environmental laws, implementing further wastewater treatment, identifying pollution sources, developing mitigation strategies, and conducting regular monitoring. Measures should be taken to reduce the release of hazardous chemicals from wastewater treatment plants into the Rosetta Nile branch. The study confirmed that consuming Nile tilapia from the Rosetta branch does not pose a significant health risk, except for antimony, which had a Target Hazard Quotient (THQ) greater than 1. Future research should investigate trace element bioaccumulation in the food chain, conduct a comprehensive health risk assessment, examine the combined effects of multiple elements, develop a predictive model for contaminant levels, and engage local communities to promote ecosystem health and sustainable practices.

CRediT authorship contribution statement

Mahmoud Al-Sisi: Writing – original draft, Formal analysis, Conceptualization. **Nevien Elhawat:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Tarek Alshaal:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Fawzy Eissa:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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