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Risk Assessment of Heavy Metal Pollution in *Oreochromis niloticus* from the Rosetta Branch of the River Nile, Egypt

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ABSTRACT

During the last three decades, an annual proliferation in the levels of heavy metals was detected in the River Nile due to sewage, industrial and agricultural wastewater discharged into the river waters. Thus, the current study was set with the aim of investigating the possible health risks associated with water use and the consumption of *Oreochromis niloticus* via comparing and determining various exposure pathways and potential health risks corresponding with heavy metals in surface water and fish off the River Nile using a number of indices. S1: El-Qanater El-Khayria site (S1; reference clean site) prior to the bifurcation, S2: El-Rahawy drain discharge point into the Rosetta Branch, and S3: El-Qatta site (about 7 km from S2) were the three locations covered by the present investigation throughout winter and summer of 2023. Heavy metals' levels were addressed (Fe, Cr, Cd, Zn, and Cu). Among the metals under research, Fe had the highest concentration, followed by Zn> Cu> Cr> Cd, and S2 had the highest heavy metal load over the course of the study. In contrast to the FAO guidelines for irrigation water and the USEPA guidelines for drinking water, water samples from S1 of the River Nile were suitable for both uses during the study, despite the fact that the heavy metal evaluation index (HMEI) of water samples taken from S2 and S3 of the Nile indicated that both sites are unsuitable for irrigation and drinking. *O. niloticus* off site S3 was monitored for non-carcinogenic health concerns in accordance with USEPA recommendations. However, chromium in fish at S3 exhibited higher lifetime potency of cocarcinogenic risks, particularly for habitual consumers. The current findings confirm the necessity of rapid water quality management planning, especially in heavily polluted areas, to secure safe water usage and maintain ecological balance and human well-being. These results also emphasize the importance of understanding the site-specific cancerogenic risks related to exposure to heavy metals in different locations and consumption patterns to appraise aquatic food safety and health risk assessments.

INTRODUCTION

Environmental pollution has been ranked as a critical problem in the 20th century, which requires national and international incorporated efforts to be encountered **(Azab *et al*., 2019)**. Most environmental pollutants threaten the welfare of humans and the environment, as well as ecological productivity and integrity **(Said *et al*., 2021)**. The previous authors attributed the increase in chemical contamination to excessive anthropogenic activities. Unfortunately, this kind of pollution has a great impact on both aquatic and terrestrial environments.

Globally, water pollution has become a problem of high concern, which negatively impacts water's quality and ceases its benefit **(Ali *et al*., 2016)**. There are seven primary groups of contaminants that can contaminate water: inorganic, organic, radioactive materials, sediments, suspended solids, heat, and heavy metals **(Botkin & Keller, 2008)**.

Water bodies, specifically rivers are markedly polluted since they receive urban, rural, and industrial wastes that usually contain high concentrations of hazardous pollutants (**Zyadah, 1999; Azab *et al*., 2019)**. The Nile is the principal body of fresh water in Egypt **(Hashem *et al*., 2020)**. It runs through the narrow valley, then bifurcates forming two branches, the Damietta and the Rosetta, forming the Delta **(Abdel-Satar *et al*., 2017)**. Currently, various challenges have encountered the Rosetta Branch. Three essential sources of pollutants have been detected releasing their effluents into the branch under estimation. El-Rahawy drain, where agricultural and domestic discharges are being released from the Giza City, forms the 1st source. Effluents discharged are estimated to be more than 1,900,000m^3 /day. Another source of pollutants originated from the industrial region of Kafr El-Zayat, while pollution spotted from numerous tiny agricultural drains, with their wastes discharged into the Rossetta Branch, has been assessed to be the third source. Furthermore, the sewage released into the branch of several cities and villages has added to the effluents received through the third source **(El Bouraie *et al.*, 2011)**.
The Rossetta Branch and its aquatic organisms are facing serious problems, among which is the heavy metal pollution **(Darweesh *et al*., 2019)**. The previous authors elucidated that the heavy metal levels' annual ratio has experienced an increase in the River Nile. The high ratio of heavy metals in the habitat is caused by anthropogenic and natural activities, among which are sewage, agricultural and industrial wastewater, microplastics, organic fuel combustion, pesticides, and phosphate fertilizers **(Said *et al*., 2021)**. Heavy metals are common harmful pollutants that damage people, plants, water, and the air these days **(Huang *et al*., 2020; Khattab *et al*., 2021, Gouda & Taha, 2023; Taha & Gouda, 2023)**. Once in the water, these toxic, bioaccumulating and non-biodegradable materials precipitate on sediment particles and build up in the food chain by damaging the organs and tissues of aquatic life **(Dural *et al*., 2006; Qiu, 2015; Mohamad *et al*., 2020; Younis *et al*., 2024b)**. It is significant to mention that the main sources of heavy metal accumulation are food and water, followed by muscles and gills **(Awad *et al*., 2024)**. It was discovered that, in addition to aquatic animals' feeding patterns, the pH, temperature, and heavy metals concentrations all affect the buildup of heavy metals **(Ali *et al*., 2016)**. The two categories of heavy metals are essential and non-essential. The vital role that critical heavy metals like Cr, Zn, Fe, Mn, and Cu play in biofunctions makes them vital for living things. Due to the harmful effects of both excess and deficiency of the important heavy metals, they are advised for this purpose at low quantities **(Santos *et al*., 2014)**. Heavy metals that are not necessary, like Pb, Hg, and Cd, have no biological purpose in living things; they are only needed in minimal amounts and turn poisonous at high concentrations **(Gati *et al*., 2016)**. The estimated 23.3% of the nation's total natural source production is derived from fish produced in the River Nile **(GAFRD,**

2015). Being at the top of the aquatic food chain, eating fish would have a harmful impact on people since fish organs and tissues accumulate heavy metals **(Abdel-Mohsien & Mahmoud, 2015)**. According to **Mohamed *et al*. (2020)**, fish can therefore be employed as bioindicators for the identification of aquatic contamination.

Because of their low cholesterol, sufficient omega-3 fatty acids in addition to other beneficial nutrients, fish are good for reducing the rate of cholesterol, the threat of heart disease as well as stroke in people. Thus, an increase has been detected in the consumption of fish **(Knuth *et al*., 2003; Abdel-Mohsien & Mahmoud, 2015)**. Accordingly, the accumulation of heavy metals in fish and other aquatic animals can be thought of as a closed circuit, with the resultant negative health effects on humans, including kidney diseases, cancer, mental illness, developmental abnormalities, and neuromuscular issues **(Zhuzzhassarova *et al*., 2024)**.

O. niloticus is the fish species most frequently found in the River Nile. For the Egyptians, it is highly popular, being the cheapest compared to other species and available all the year- round **(Abdel-Mohsien & Mahmoud, 2015; El-Naggar *et al*., 2021)**. *O. niloticus* is capable of tolerating deteriorated environmental conditions due to its high physical resistance and slight respiration demand, allowing it to withstand ammonia with high amounts in addition to low oxygen **(Zhou *et al*., 1998)**.

With the aim of addressing heavy metal pollution in the Rosetta Branch of the River Nile and the accumulation of these metals in *O. niloticus*, the present investigation was conducted. In addition, the study focused on determining the validity of water for both drinking and irrigation, as well as assessing the potential health risks in relation with oral and dermal contact of water and the ingestion of the Nile tilapia from the River Nile.

MATERIALS AND METHODS

1. Study area

The Rosetta Branch of the Nile River is roughly 225km long, 150–200 m wide, with an average depth of 2.0– 3.5m. Every day, the branch receives and discharges drainage water from agricultural areas, as well as partially treated and untreated domestic and industrial wastewaters with cubic meters of 3 million or more **(Gaber *et al*., 2013)**. It terminates at the Rosetta Estuary after beginning at El-Qanater El-Khayria. El-Rahawy, the Zawyet El-Bahr, El-Tahrer, the Tala, and the Sabal, which are the five agricultural drains that discharge into the branch **(Donia, 2005)**. The present study focused on El-Rahawy drain, which is an extremely big drain receiving industrial, agricultural, and domestic wastes of Giza City. It discharges more than 1,9 million m^3/day as sewage waste directly into the Rosetta Branch.

The inquiry study area spans over 20 kilometers from the El-Qanater El-Khayria site to the El-Qatta site. Three locations were the subject of the current study: S1: El-Qanater El-Khayria site (reference clean site) before to the bifurcation; S2: El-Rahawy drain discharge point into the

Rosetta Branch (about 12.6km from S1); and S3: El-Qatta site (approximately 7km from Site 2) (Fig. 1). The latitudes and longitudes of the sites under study are displayed in Table (1).

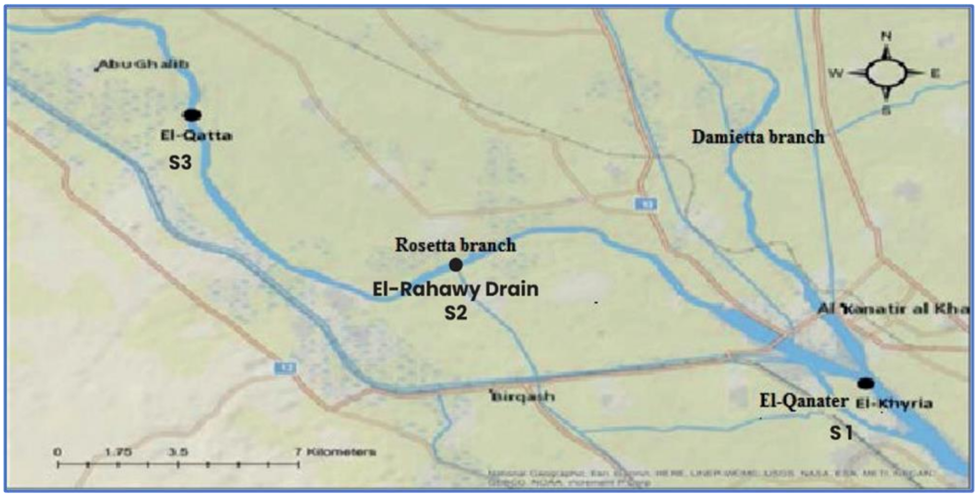

Fig. 1. The Nile Delta map showing sites under investigation **(Mohamed *et al*., 2020)**

Table 1. Longitudes and latitudes of sites under study (GPS)

Site	Location	Latitude	Longitude
S1	El-Qanater El-Khayria (before the bifurcation)	30° 10' 19.0344" N	31° 8' 24.0252" E
S2	El-Rahawy drain (discharge point)	30° 12' 20.0988" N	31° 1' 40.962" E
S3	Al-Qatta site (downstream EL-Rahawy drain)	30° 13' 27.6636" N	30° 58' 26.1444" E

2. Samples collection

2.1. Water sampling

Under the code NIOF-FW3-F-23-R-003, the current study was conducted in compliance with the ethical requirements of the National Institute of Oceanography and Fisheries' (NIOF, Egypt) committee for the ethical care and use of animals/aquatic creatures. Throughout two seasons in 2023 (winter & summer), samples from surface water were taken from the three study locations, down to a depth of 30cm.

2.2.Fish sampling

Thirty *O. niloticus* specimens, regardless of their sex, with a mean total length of 20.03± 1.62cm and mean total weight of 163.19± 24.08 g were sampled during the study period, with the help of fishermen available at two sites: S1 S3 (affected by El-Rahawy drain) with the help of fishermen. No fish were found alive during winter at S2, El-Rahawy drain discharge point.

3. Determination of HM

3.1. HMs analysis in water

For heavy metals analysis, the flame atomic absorption spectrophotometer (Savant AA, GBC Scientific Equipment) was utilized in the Central Laboratory, Faculty of Science, Ain Shams University in accordance with the American Public Health Association's Standard Method (APHA-3111B, 2017). The findings of the analysis of five heavy metals (Zn, Cu, Cd, Fe, and Cr) were expressed in µg/L.

3.2. HMs analysis in fish samples

According to **El-Dakar *et al*. (2021)**, with minor modifications, fish were anesthetized with 40 mg/L clove oil upon capture. The muscles, liver, and gills of the fish were then removed, chopped into tiny pieces, and kept in an ice box until they were sent to the Central Laboratory, Faculty of Science, Ain Shams University, for the measurement of the aforementioned heavy metal levels.

According to **El-Naggar *et al*. (2022)**, the subsequent procedure was implemented: The CEM microwave sample preparation equipment (MDS-2000, USA) was employed to digest the samples before analysis. Nitric acid (HNO_3) in a concentrated form was added to containers containing one gram of ground material.

For an adequate reaction, the vessels were left overnight and later, a turntable, where vessels were placed, was attached to the system and a heating program was implemented. Vessels were set to run till digestion was completed. Afterward, samples cooled down for 5min, followed by separating the turntable from the system.

For the heavy metals analysis, the digested samples were merged into 25ml of distilled water and were placed in the flame atomic absorption spectrophotometer. Each heavy metal concentration was measured at a specific slit width and wavelength. The resultant concentration was expressed in mg/kg of the tissue.

4. Risk assessment

4.1. Health hazard assessment for water uses

4.1.1 Water samples' heavy metal assessment index (HMEI)

Heavy metal (HM) concentrations are evaluated using HMEI to determine the water quality's numeric value, adhering to the permissible standard of the same HM for irrigation **(FAO, 2019)** and drinking **(USEPA, 2018)**. This step was accomplished using **Singh *et al*. (2017)**'s equation. When HMEI is less than 1.0, water samples are considered felicitous for human use; if HMEI is greater than 1.0, they are considered unsuitable.

4.1.2 The daily chronic intake (CDI)

CDI was estimated as stated by **USEPA (2004)** for dermal adsorption and oral ingestion of HM detected in water samples of the River Nile.

4.1.3 Target hazard quotient (THQ)

The THQ is considered to compute the interplay of HM exposures through water and identify the safety threshold, which doesn't persuade antagonistic health impacts on humans, conforming the equations of **USEPA (2004)** and the reference dose guidelines of each metal (mg/Kg Day) for the dermal contact and ingestion of water established by **USEPA (2011)**.

4.1.4 Hazard index (HI)

The HI is the accumulation of total THQ; it reveals the potential non-carcinogenic health hazards encouraged by HM in water following **USEPA**'s **(2004)** equations. HI ≤ 1.0 reflects the anticipation of no harmful healthiness consequences.

4.2. Assessment of Health hazard for fish consumption

4.2.1 Estimated daily intake (EDI) of HM

EDI was estimated for adults (70Kg) using the outline ingestion rates of fish (habitual & normal) covering a lifespan of 70 years, as detailed in the research works of **Song *et al*. (2009)** and **Salaah *et al*. (2022)**.

4.2.2 Target hazard quotient (THQ)

The THQ is generally conducted to assess the non-carcinogenic hazards of every single consumed metal by means of the metal's reference dose stated by **USEPA (2011, 2012)**.

4.2.3 Hazard index (HI)

HI is the metals' collective THQ. It presents the comprehensive health risk owing to vulnerability to several metals **(USEPA, 2011)**.

4.2.4 Target cancer risk (TR)

According to **USEPA (2011)**, TR is obtained to evaluate the likely carcinogenic risk in correspondence with the utilization of specific HM (Cd Cr) from fish. At ≤ 1.0 ×10-6, ΣTR and TR are considered insignificant **(USEPA, 2010)**.

5. Statistical analysis

The season and site efficacy on the heavy metal bioaccumulation in the tissues of *O. niloticus* were ascertained by employing Jeffreys's Amazing Statistics Program (JASP 0.16.4) to perform a two-way analysis of variance (ANOVA) on the data of the heavy metal levels in the tissues. Three separate analyses were conducted. Tukey's test **(Abdi & Williams, 2010)** was used to elucidate the differences between the specimens under study.

RESULTS AND DISCUSSION

1. Heavy metals concentrations in water

Heavy metals' average concentrations detected in the samples of surface water at the studied sites throughout the study period are shown in Fig. (2). Generally, they were determined in a descending order, as follows: Fe, Zn, Cu, Cr, and Cd.

As shown in Fig. (2A), the three sites' iron concentrations ranged from a maximum average value of 1200µg/ L during winter at S2 to a minimum mean value of 200µg/ L during summer at S1. According to the **CCME (2017)**, the iron levels in the water at the three sites exceeded the threshold limit of 300µg/ L, except for the Fe level that was recorded during summer at S1. The aforementioned findings coincide with the outcomes of **Hashem *et al*. (2020)** and **Mohamed *et al.* (2020)**, who illustrated that the concentrations of Fe at Sites 2 and 3 surpassed the allowable levels. The phytoplankton's utilization of iron **(Canli & Kalay, 1998)**, oxidation of Fe2+ to Fe3+, and subsequent precipitation of hydroxide an elevated dissolved oxygen content may be the reasons why the lowest concentration of Fe was detected at S1 during summer **(Ghallab, 2000)**. S2 and S3 had the greatest mean Fe concentrations in both seasons (1200 and 950µg/ L, respectively), with S3 having the highest concentration in winter (900µg/ L). The outflow of effluents from El-Rahawy drain, being filled with both domestic and agricultural sewage, may be the cause of the high Fe content at sites 2 and 3 **(Haggag, 2017)**. Furthermore, according to **El-Sayed and Salem (2015)**, this high value can be a result of the release of this heavy metal into the water through the breakdown of organic matter and dead microbes. **Abdelsalam *et al*. (2024)** added that the lowest concentration of iron during summer is due to the suspending of iron particles, surface microorganisms, presence of metal oxides owing to high temperature.

At the current three sites under study, zinc concentrations varied from a minimum average concentration of 80µg/ L during the summer at S1 to a maximum mean level of 200µg/ L during winter at S2 (Fig. 2B). Notably, the **CCME (2017)** found that the levels of zinc in the water of the three research locations were higher than the standards. The results presented above are consistent with those of **Hashem *et al*. (2020)**, who hypothesized that zinc amount at sites 2 and 3 was higher than the allowed. Prior to S3 (120µg/ L) in winter, S2 under investigation had the highest zinc level (200 and 130µg/ L, respectively). **Abdel-Satar *et al*. (2017)** suggest that the impact of anthropogenic activities could be the cause of this elevated concentration at S2. One of the most common contaminants found in waste and water, both in liquid and solid form, is zinc. Furthermore, due to its acute toxicity and lack of biodegradability, zinc is classified as a hazardous waste **(Younis *et al*., 2024 a)**. Accordingly, **Abdelsalam *et al*. (2024)** ascribed the increasing Zn concentration in winter to the drought period, when the water level drops, which in turn increases the microbiota activity and causes organic drips to bioaccumulate. These organic drips may act as humic acids that contain a variety of heavy metals, such as Cu, Zn, Fe, Cd, Pb, and Mn. **Ezzat *et al*. (2024)** ascribed the elevated Zn concentration in aquaculture to the drainage of agricultural and industrial wastes into the water.

Data denoted in Fig. (2C) show that the highest wintertime concentration of Cu was recorded at S2 (55µg/L), followed by S3 (40µg/ L). It was noted that during the summertime, the Cu content at S1 was less than the standards of the **Egyptian Governmental Law no. 48/1982-Decision 92 (2013)** (6µg/L)). The tendency of Cu to form complexes with humic matter and organic ligands, eliminating free ions' penetration into the water, is thought to be the cause of the lowest Cu level in water during summer at the studied sites **(Mantoura *et al*., 1978; Moustafa, 2017)**. For Cu levels, they surpassed the acceptable levels during winter at S2 & S3, whereas they were within the thresholds of the **Egyptian Governmental Law no. 48/1982- Decision 92 (2013)** at S1 in summertime (10 µg/ L). Accordingly, the previous findings coincide with those of **Hashem *et al.* (2020)** and **Mohamed *et al*. (2020)**, who revealed that the Cu levels at Sites 2 and 3 exceeded the standard limits. The aforementioned data may be ascribed to the decline in the level of water in the River Nile during the rainy season as well as the rise in domestic waste at El-Rahawy drain, whose discharge will affect the metal's level at S3.

Notably, the Cr concentration in water at S1 was within the quality standard values of the **CCME (2017)** (10 µg/L). Cr concentrations at the investigated sites ranged between a maximum mean value of 26µg/ L during winter at S2 and a minimum average value of 7µg/ L during the dry season at S1 (Fig. 2D). It was noticed that Cr concentration exceeded the thresholds of the **CCME (2017)** at both sites (2 & 3) during both seasons (Fig. 2D). It was annotated that heavy metals' levels in the investigated samples showed season-specific variations, being the highest during the winter months **(Abdel-Moati & El-Sammak, 1997)**. This may be due to the increase in the amount of industrial, agricultural, and sewage wastes that are discharged into the River Nile during winter **(Khalil *et al*., 2017)**. Conversely, the decrease of the HM contents in summer may be interpreted as an outcome of the phytoplankton's development, which can absorb significant amounts of HM from water, in addition to the rise in the River Nile's water level throughout the summer season **(Tayel *et al*., 2018)**.

Cd concentrations at the sites under investigation ranged between a maximum average value of 12µg/ L during winter at S2 and a minimum mean concentration of 0.5µg/ L during summer at S1 (Fig. 2E). All Cd values at the studied sites exceeded the standards recommended by the **CCME (2017)** and the **Egyptian Standards of the Environmental Laws no. 48/1982 decree 92/2013-Decision 92 (2013)** (1 µg/L), except for those observed during summer at S1. The previously mentioned findings may account for the effect of pollution sources in the locations under study, such as sewage and landfill at S3, residential wastes at S2 and anthropogenic activities; mainly agricultural at S1 that are especially amplified during the rainy season.

In their study (**El-Degwy *et al*., 2023)**, an association was recorded between the elevated levels of Zn and Cd in the water of Lake Mariout and the high temperatures and fermentation during summer, with the former subsequently releasing from the bottom sediment into the water column above.

They concluded that all the investigated heavy metals' concentrations are greater in the rainy season compared to the dry one. Furthermore, the fluctuations in the levels of the heavy metals (Fe, Zn, Cu, Cr & Cd) at the studied sites may be ascribed to the dynamic variation of wastewater flow in the area or the time of water sample collection. Such seasonal variation of heavy metal levels in water is traced back to the variation of domestic, industrial and agricultural activities, Moreover, such differences may be related to the hazard impacts of varied pollutants, viz. residential, agricultural, and industrial ones (**El-Degwy *et al*., 2023)**.

2. Heavy metals' bioaccumulation in various tissues of *O. niloticus*

Fish are commonly used as biological indicators to assess the level of metal contamination in aquatic environments **(Al-Sayegh Petkovšek *et al*., 2012)**. This is confirmed since they can accumulate high concentrations of certain metals found in the water **(Daviglus *et al*., 2002)**. When contaminated fish are consumed, toxic elemental pollutants are introduced into the human body, which can lead to a significant decline in health **(Alinnor & Obiji, 2010)**. Therefore, consuming fish in amounts exceeding the recommended safety levels can be extremely harmful to humans **(Basiony, 2014)**.

Heavy metals are known to aggregate in the tissues of fish. Through direct ingestion from the sediment and water or indirect ingestion throughout the food chain, they may acquire metals. These metals are subsequently built up in fish tissues and organs in amounts greater than those in their environment, which might have negative consequences **(Agah *et al*., 2009)**. Based on data in the study of **Neima *et al*. (2016)**, heavy metals usually form cationic complexes built up in the internal organs of fish. According to **Neima *et al*. (2016)**, the accumulation of heavy metals is often impacted by the concentration of the metal, the mode of metal uptake, the duration of exposure, intrinsic characteristics (like fish age and eating habits), and environmental factors (viz. salinity, temperature, and pH). Due to variations in permeability, metabolic rates, and the types and quantities of metal-binding ligands on the organism's surface, this buildup differs with the species, the type of heavy metal, and even the individual **(Campenhout *et al*., 2004; Javed & Usmani, 2019)**.

An understanding of the relationship between fish and their external environment can be gained from the accumulated metals in key organs, mirroring the concentrations of these metals in the fish species' surrounding environment **(Monroy *et al*., 2014)**.

2.1. Heavy metal contents in the gills tissues of O. niloticus

The mean concentrations of HMs in the gills samples of O. *niloticus* off different sites in the River Nile covering the period of work are depicted in Table (1). At both sites, the average levels of heavy metals in the gills were in the descending order of Fe> Zn> Cu> Cr> Cd.

Remarkably, site- as a factor- was recorded with a significant (*P*< 0.05) effect for HMs under investigation, monitoring high significant mean values at S3 compared to those recorded at S1. This means that the gills, due to their anatomical position, showed high levels of some metals' accumulation patterns, since they are continuously and directly affected by the surrounding contaminants **(Abdel-Khalek *et al*., 2016)**.

Moreover, the levels of Fe, Zn, Cu, and Cr were recorded to be significantly (*P*< 0.05) impacted by site and season. Their highest significant values were recorded at S3 during winter (133.97, 45.12, 4.31, and 2.76µg/ g, respectively), while their lowest significant levels were found at S1, during summer (46.00, 23.79, 2.09 and 1.11µg/ g, respectively). On the other hand, neither the season nor the interaction between the two factors exhibited a crucial effect on the values of Cd, though they numerically differed. Furthermore, it was noted that the average values of Fe and Zn at S3 during winter (133.97 and 45.12µg/ g, respectively) exceeded the threshold of the **WHO (1993)** (100 and 40µg/ g). Whereas, the mean concentrations of Cr at both sites during both seasons surpassed the permissible level recommended by the **FAO (1983)** (1µg/ g). Whereas, Cd levels at S3 during both seasons (winter & summer) outreached the permissible dose reported by the **WHO (1993)** (0.5µg/ g).

In general, the increment of the addressed heavy metals in gills is associated with their elevated concentrations in water originated from the flow of huge quantities of sewage waste into the studied area. These results may be attributed to the following reasons: 1) It is well known that gills are the primary site that create a pathway for heavy metals to go into the fish's body **(Bols *et al*., 2001)**; 2) Gills, being directly exposed to the environment, are known for their potential to excrete certain metals viz. lead **(Matthiessen & Brafield, 1977)**; 3) **Wepener *et al.* (2001)** noted that gills are the first site where waterborne metals accumulate owing to their bonding with gill cytosolic chemicals, and 4) the metals absorbed onto the gill surface, which serves as the initial point of contact for contaminants in the water, is another reason. Furthermore, according to **Ezzat *et al*. (2024)**, Zn is highly accumulated in the body of *O. niloticus* in Burullus Lake due to the highly effective uptake and storage through fish gills and internal organs. In contrast with our observations, the previous authors detected a relation between the summer season and the increase in pathological changes and weakness of gills. They deduced that the following pathological changes are traced back to the elevated concentrations of heavy metals throughout summer: gill damage, gas exchange disruption and increasing gills inflammatory responses causing pathogen growth and activation within the gills.

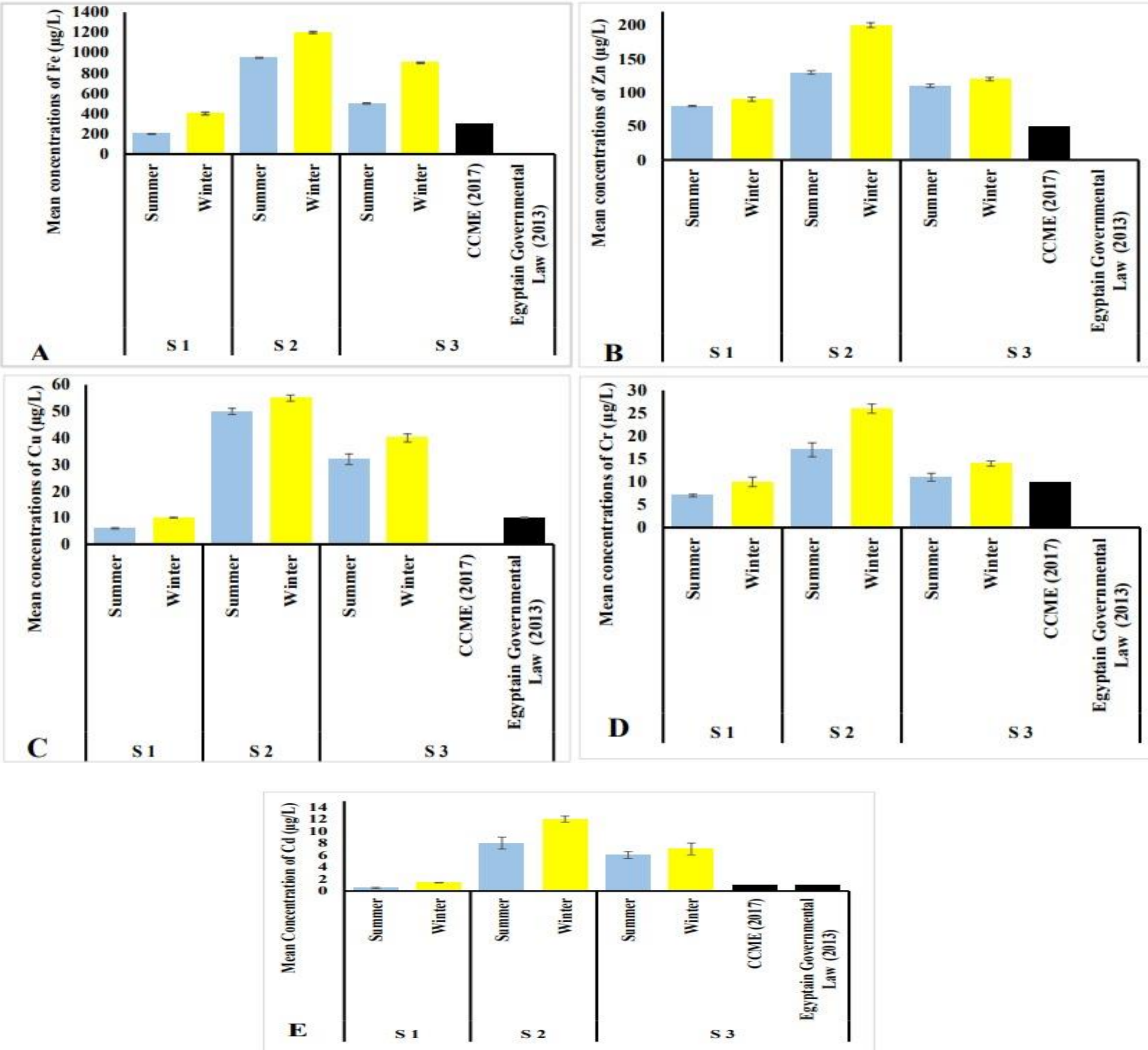

Fig. 2. Mean concentrations of HMs in samples of surface water at the investigated sites throughout the study period including (A) Fe, (B) Zn, (C) Cu, (D) Cr. and (E) Cd

S1: El-Qanater El-Khayria (non-polluted site, reference site). S2: El-Rahawy drain (discharge point). S3: Al-Qatta site (polluted site)

2.2 Levels of heavy metals in O. niloticus liver tissues

Table (2) lists the average levels of heavy metals detected in the Nile tilapias' liver samples, with specimens from several locations. In general, liver was recorded with the largest amounts of most heavy metals. This can be confirmed because the liver substantially induces metallothionein and stores redistributes, detoxifies while converting contaminants **(Yılmaz *et al*., 2007)**. The liver is a highly active organ that is specialized in absorbing and storing a variety of heavy metals **(Dural *et al*., 2006; Yılmaz *et al*., 2007)**. Furthermore, the liver tissue's metabolic activity makes it quite likely that most metals will be concentrated in large quantities within its tissues **(Omar *et al*. 2014)**. The average heavy metal levels in the 1iver at both sites are sequenced as follows: Fe> Zn> Cu> Cr> Cd. It is worth mentioning that all heavy metals were significantly affected by site and season ($P < 0.05$), in which their highest significant values were recorded at S3 during winter, while their lowest were noted during summer at S1.

Table 1. Mean concentrations of heavy metals (µg/ g) in *O. niloticus* gills from different sites throughout the study period

	Site per season						
	S1		**S3**		**Site effect (*P*-value)**	**Season effect (*P*-value)**	**Permissible dose for *O. niloticus***
Heavy metal	**Summer**	**Winter**	**Summer**	**Winter**			
Fe	46.00±4.17^{d}	61.04 ±1.8^{c}	96.50± 3.16^{b}	133.97± 0.551^{a}	< .001	< .001	100
Zn	23.79 ± 0.42^{d}	30.60± 0.23^{c}	37.54± 1.21^{b}	45.12± 1.42^{a}	< .001	< .001	40
Cu	2.09 ± 0.008^{d}	2.59± 0.169^{c}	3.82± 0.05^{b}	4.31±0.04^{a}	< .001	< .001	30
Cr	1.11± 0.018^{c}	1.59± 0.02^{b}	1.35± 0.14^{bc}	2.76± 0.092^{a}	< .001	< .001	1
Cd	0.09±0.007	0.15± 0.004	0.52±0.32	0.73±0.010	0.013	0.447	0.5

Each value illustrates the mean level± SE, SE: Standard error (n=3).
Values in the same row with varied superscripts are significantly different ($P < 0.05$) by Tukey's test.
All the permissible doses are according to **WHO (1993)** except that of Cr which is in accordance with **FAO (1983)**.
S1: El-Qanater El-Khayria (non-polluted site, reference site).
S3: Al-Qatta site (polluted site).

Moreover, it was noticed that the mean concentrations of Fe at S3 during both seasons were 140.65 and 277.67µg/ g, respectively, whereas at S1 during winter (138.39µg/ g), values surpassed the permissible limits reported by the **WHO (1993)** (100µg/ g). On the other hand, the average values of Zn at S3 and S1 during winter (65.89 and 42.00µg/ g, respectively) exceeded the threshold of the **WHO (1993)** (40µg/ g). However, the mean value of Cu at S3 during winter (53.33µg/ g) exceeded the threshold determined by the **WHO (1993)** (30µg/ g). Whereas Cr levels outreached the threshold reported by the **FAO (1983)** (1µg/ g) at both sites during both seasons. Nevertheless, Cd values at S3 during both seasons (0.64 and 0.84µg/ g, respectively) exceeded the recommended limits assigned by the **WHO (1993)** (0.5µg/ g). Cd is a serious environmental pollutant that can cause detrimental effects on fish, such as growth deficits **(Peterson *et al*., 1983)**, biochemical effects **(Haux & Larasson, 1984)**, and osmoregulatory problems **(Reid & McDonald, 1988)** at sublethal doses or slightly high doses **(Abdel-Mohsien & Mahmoud, 2015)**. The elevated levels of Cd may be ascribed to wastes discharged from mining and industrial operations, phosphate fertilizer, and sewage at El-Rahawy drain, where almost all Cd concentrations are accumulated in the habitat **(Mohamed *et al*., 2020)**.

Major histopathological alterations were induced in the liver of the Nile tilapia by the biomagnification of heavy metals (Cd, Fe, Cu, Zn, and Pb) in Burullus Lake, such as necrosis, pyknosis, and vascular damage of hepatocytes (**Ezzat *et al*., 2024)**. The aforementioned pathological changes are assigned to the seasonal changes, i.e. during summer, heavy metal content and algae bloom increase in the water, and accordingly the energy requirement increases, causing oxidative stress and liver damage, which in turn reflects on fish health.

Table 2. The average concentrations of heavy metals (µg/ g) in the liver of the Nile tilapia obtained from the sites under study during summer and winter of 2023

	Site per season						
	S1		**S3**		**Site effect (*P*-value)**	**Season effect (*P*-value)**	**Permissible dose for *O. niloticus***
Heavy metal	**Summer**	**Winter**	**Summer**	**Winter**			
Fe	57.07± 7.78 [c]	138.39± 4.53 [b]	140.65± 0.325[b]	299.67± 1.35 [a]	< .001	< .001	100
Zn	23.75± 0.42 [c]	42.00±0.07 [b]	38.72± 0.59 [b]	65.98±1.47 [a]	< .001	< .001	40
Cu	3.44±0.55 [b]	6.96±1.67 [b]	25.70± 9.4 [b]	53.33± 3.78 [a]	< .001	0.017	30
Cr	1.13± 0.014 [d]	1.78±0.034 [c]	1.94± 0.026 [b]	2.85± 0.05 [a]	< .001	< .001	1
Cd	0.13± 0.003 [d]	0.16±0.005 [c]	0.64± 0.005 [b]	0.84± 0.005 [a]	< .001	< .001	0.5

Each value represents the average value± SE, SE: Standard error (n=3).
Values in the same row with different superscripts are significantly different ($P< 0.05$) by Tukey's test.
All the permissible doses are according to **WHO (1993)** except that of Cr which is in accordance with **FAO (1983)**.
S1: El-Qanater El-Khayria (non-polluted site, reference site).
S3: Al-Qatta site (polluted site).

2.3 Metal contents in O. niloticus muscle tissues

The average levels of heavy metals in the muscles in both locations are as follows: Fe > Zn > Cu > Cd > Cr. All of the heavy metal concentrations in the muscles were shown to be significantly impacted by site and season ($P< 0.05$); the greatest significant values were found at

S3 in winter, while the lowest significant levels were found at S1 in summer. However, neither the Zn, Cu, and Cr values across the two seasons nor the Cd value at S3 during winter (Table 3) showed any discernible changes for S1.

The levels of Zn and Fe at S3 during winter exceeded the permissible doses for *O. niloticus*. While, the levels of Cd surpassed the recommended limits **(WHO, 1993)** for the fish at both sites during both seasons. Whereas, for the safety of human consumption, all heavy metals were found within the allowable levels of the **FAO/WHO (2011)** at both sites during both seasons, except for Cd and Cr.

The summer and wintertime witnessed iron levels in fish muscles ranging from 40.83 to 54.15µg/ kg at S1 and from 90.80 to 124.52µg/ kg at S3. Remarkably, iron was the highly prevailing metal in the tissues under investigation. Iron elevation in water is likely the cause of Fe, being densely accumulated in fish muscles, especially at S3 **(Khalil *et al*., 2017)**. Data in Table (3) reveal that the iron contents in fish muscles are greater than the recommended dose of the **WHO (1993)** (100µg/ kg at S3 during winter). Besides, the largest concentration of iron detected in fish muscles may be found in the River Nile waters and in household sewage at S2. On the other hand, the lowest values at S1 might be the result of Fe^{2+} being oxidized to Fe^{3+}, which then remains as $Fe(OH)_3$ in the sediment of the oxygenated water.

Zinc was recorded with the maximum value in fish muscles at the polluted site (S3 during winter (41.66 µg/kg), while the minimum value (22.96 µg/kg) was noticed at S1 during summer. The value of Zn surpassed the permissible level of 40.0 µg/kg (**WHO, 1993)** at S3 during winter. A relation was assessed between the high uptake of heavy metals, the increase in zinc, and the great metabolic rates, previously spotted in the study of **Sorensen (1991)**. Another perception may relate the elevated levels of Zn in water at S3 to the decline in the water discharged throughout the months of winter, which subsequently increases the domestic waste in S2.

The summer and winter cadmium concentrations in fish muscles varied from 1.05 to 1.10µg/kg at S1 and from 1.07 to 2.13µg/kg at S3. The cadmium concentrations found in fish muscles surpassed the allowable threshold of **WHO (1993)** (0.5µg/kg). A significant bond between cadmium and the cysteine residue of metalothionein was previously detected in the studies of **Abu El-Ella (1996)** and **Tayel *et al*. (2018)** leading to high levels of cadmium found in fish muscles. Furthermore, the phosphate fertilizer used at S2, where most of the cadmium accumulates in the environment, along with waste from industrial, mining, and sewage activities, is probably associated with the elevated cadmium levels **(Dimari *et al*., 2008)**.

The results of **El-Degwy *et al*. (2023)** are contradictory with the current results, where the high heavy metals' levels (Fe, Mn, Cu, Zn, Cd and Ni) in the edible parts of *O. niloticus* were distinguished in summer owing to the untreated waste disposal, urban development, runoff from municipal sewage, and industrial wastes.

The present findings showed that copper, iron and zinc were the metals most dominating in fish organs. **Kumar *et al*. (2011)** analyzed these results and contended that these metals are necessary because they are regulated to preserve a particular homeostatic state in fish and act as co-factors for the activation of several enzymes. Conversely, non-essential metals are usually present in fish in trace concentrations and serve no biological purpose. For instance, the heavy metals cadmium and chromium are toxic, non-essential, and non-biodegradable. They are not involved in biological processes in living things, but they can be dangerous in small amounts. Consequently, these metals have the potential to harm fish even at low doses **(Badr *et al*., 2014)**.

Relative to other tissues, the lowest concentrations of all the heavy metals under study were detected in fish muscles. This result coincides with the finding of **Darweesh *et al*. (2019)**. Moreover, **Bahnasawy *et al*. (2010)** reported that muscles are non- biologically active organs in heavy metals accumulation.

The levels of heavy metal residues in the liver and gills of *O. niloticus* were considerably greater than those in the muscles, according to the findings of the study on heavy metals in fish tissues. Numerous authors' data **(Saeed & Shaker, 2008; Farouk, 2009; Yosef & Goma, 2011; Abd-El-Khalek *et al*., 2012; EL-Shaer & Alabssawy, 2019; Aly *et al*., 2020)** support this result. This outcome can be ascribed to fish's gill tissues and liver creating metallothionein proteins to help with detoxification when they are vulnerable to heavy metals. According to **Jobling (1995)** and **Yacoub (2007)**, these proteins are essential for shielding tissues from the detrimental influence of heavy metals.

In comparison with fish muscles, the higher levels of heavy metals in gills can be related to the muscles challenging to completely remove the heavy metals from the platelets **(Heath, 1995)**. Hence, muscles do not actively accumulate heavy metals (**Karadede & Ünlü, 2000)**. Various studies involving different fish species **(Guerrin *et al*., 1990; Alam *et al*., 2002)** reported similar findings, possibly due to the low binding affinity of the proteins in the muscles **(El-Shaer & Alabssawy, 2019)**. It may also be the cause of the trace amounts of heavy metals in fish muscles because of the scales on the fish's skin, mucous secretions, and skin barrier, which all decrease the amount of water that carries heavy metals to the body through the muscles and skin.

Fish liver is a reliable indicator of contamination in the aquatic environment since it has a vital role in metabolizing and excreting xenobiotic compounds **(Rocha & Monteiro, 1999)**. In addition, the liver filters the contaminated blood that is transported through the intestine. Additionally, **Habib *et al*. (2023)** and **Lawan & Akawu (2024)**, in their studies on *O. niloticus*, attributed the highest quantities of heavy metals to the liver, being the main organ responsible for heavy metal accumulation for its function in the detoxification and removal of heavy metals from the body, due to its high metallothionein protein content, which has a great ability to bind with heavy metals and to prevent their circulation.

The current results indicate that fish living in aquatic habitats with high metal ion concentrations tend to absorb these ions into their tissues in various ways. Metal concentrations in natural aquatic environments are typically low, ranging from nanograms to micrograms per liter. Fish living in these environments primarily accumulate metals through their diet and water **(Bury *et al*., 2003)**. In this context, metal accumulation was observed in *O. niloticus* under investigation at significantly higher concentrations compared to those recorded in the surrounding water. The increased metal accumulation in the muscles of the species under investigation at S3 occurred during winter, and this elevation can be ascribed to the low water levels during the drought and the high levels of domestic sewage discharged into S2.

3. Health risk assessment

3.1. Oral and dermal exposure of the River Nile

In this study, the HMEI was assessed for both drinking and irrigation water quality standard of water sampled from three localities throughout the period of investigation (winter and summer of 2023). S1 recorded had appropriate HMEI values for both irrigation and drinking uses, with values that ranged between 0.79 and 0.24 in winter, and 0.62 and 0.21 in summer, respectively. These values categorized the water at S1 as 'Fit' for both uses during the study, as demonstrated in Table (4).

On the contrary, S2 and S3 exhibited significantly higher HMEI values, indicating 'Unfit' conditions for both drinking and irrigation. In winter, the HMEI values for drinking and irrigation were higher than summer, confirming the persistent and severe contamination at these sites, especially in winter.

Table (5) shows seasonal and site-based variations of the daily intake (EDI) of heavy metals evaluated for *O. niloticus* adults through both oral ingestion and dermal absorption at various stations. For oral ingestion, Fe displayed the highest EDI among estimated metals, with values reaching 3.77E-05 and 2.98E-05 mg/kg/day in winter and summer at S2, respectively. Conversely, Cd had the least metal EDI, recording 2.20E-08 and 1.57E-08 mg/kg/day in winter and summer at S1, respectively. Likewise, for dermal absorption, Fe presented the highest EDI, with 1.85E-07 and 1.47E-07 mg/kg/day in winter and summer at site S2, correspondingly. Cd had the lowest EDI, measured at 1.08E-10 and 7.71E-11 mg/kg/day in summer and winter at site S1. Basically, Fe had the highest EDI among metals for both paths, while Cd had the lowest EDI, with substantial differences among sites across seasons. Throughout the different pathways and seasons, S2 exhibited the highest EDI for heavy metals, and the lowest displayed at S1. Generally, the EDI values for most heavy metals were lower in summer than winter, signifying a potential seasonal effect on pollutant levels.

Table 3. The heavy metals mean levels (µg/ g) in the Nile tilapia's muscles gathered from the studied sites during summer and winter of 2023

	Sites per season							
	S1		**S3**		**Site effect (*P*-value)**	**Season effect (*P*-value)**	**Permissible dose for *O. niloticus* (µg/ g)**	**Permissible dose for humans (µg/ g)**
Heavy metal	**Summer**	**Winter**	**Summer**	**Winter**				
Fe	40.83± 0.3 [d]	54.15± 1.5 [c]	90.80± 0.793 [b]	124.52± 1.095 [a]	< .001	< .001	100	425
Zn	22.96± 0.58 [c]	24.89± 0.76 [c]	34.35± 1.088 [b]	41.66± 0.63 [a]	< .001	< .001	40	99.4
Cu	1.22± 0.072 [c]	1.30± 0.15 [c]	2.26± 0.14 [b]	3.36± 0.088 [a]	< .001	0.001	30	50
Cr	0.09± 0.026 [c]	0.13± 0.05 [c]	0.50± 0.06 [b]	0.66± 0.085 [a]	< .001	< .001	1	0.5
Cd	1.05± 0.002 [b]	1.10± 0.0005 [b]	1.07± 0.008 [b]	2.13± 0.029 [a]	< .001	< .001	0.5	1.3

Each value designates the mean value± SE, SE: Standard error (n=3).
Values in the same row with different superscripts are significantly different ($P < 0.05$) by Tukey's test.
The permissible doses for the fish are according to **WHO (1993)**, except Cr which is in accordance with **FAO (1983)**.
The permissible limits for human consumption are in accordance with **FAO/WHO (2011).**
S1: El-Qanater El-Khayria (non-polluted site, reference site).
S3: Al-Qatta site (polluted site).

The non-carcinogenic health risks of HM via different pathways of exposure of the Nile water samples covering the investigated seasons are demonstrated in Table (6). Across the studied seasons, the greatest oral THQ values were observed at S2 for Cd followed by Cr; whereas, the lowest THQ values were documented at S1 for Fe and Cu. Concerning the dermal pathway, the highest THQ values were recorded in winter for Cd at S1 followed by Cr at S2, while the lowest THQ values were noted at S1 for Fe succeeded by Cu at S1. In summer, the THQ highest values were found at S2 for Cr at S2 followed by Cd, with the lowest THQ values at S1 for Cu and Fe.

The HI for oral pathway displayed the highest value in winter at S2 and in summer at S2. While, both winter and summer witnessed the most elevated HI values at S2. The HI values across seasons exhibited no non-carcinogenic health risks related to different exposure pathways of heavy metals from the water of the Rive Nile.

Table 4. Heavy metal evaluation index (HMEI) values for water sampled from the River Nile during winter and summer of 2023

	Winter (HMEI)				Summer (HMEI)			
	(Drinking quality)		(Irrigation quality)		(Drinking quality)		(Irrigation quality)	
	Value	Description	Value	Description	Value	Description	Value	Description
S1	0.79	Fit	0.24	Fit	0.62	Fit	0.21	Fit
S2	6.74	Unfit	2.08	Unfit	5.00	Unfit	1.47	Unfit
S3	4.59	Unfit	1.28	Unfit	3.02	Unfit	1.03	Unfit

Table 5. Estimated daily intake (EDI) of heavy metals for adults via dermal and ingestion absorption of water sampled from the Nile River during the study

EDI $_{Oral}$										
	Winter					Summer				
	S1	**S2**	**S3**	**Mean**	**S. D**	**S1**	**S2**	**S3**	**Mean**	**S. D**
Fe	5.34E-06	3.77E-05	2.82E-05	2.3E-05	1.66E-05	4.08E-06	2.98E-05	1.57E-05	1.66E-05	1.29E-05
Cu	2.51E-07	1.72E-06	1.25E-06	1.07E-06	7.54E-07	1.88571E-07	1.57E-06	1.03E-06	9.32E-07	6.97E-07
Zn	1.88E-06	6.28E-06	3.77E-06	3.98E-06	2.20E-06	2.51429E-06	4.08E-06	3.45E-06	3.35E-06	7.90E-07
Cd	2.20E-08	3.77E-07	2.20E-07	2.06E-07	1.77E-07	1.57E-08	2.51E-07	1.88E-07	1.51E-07	1.22E-07
Cr	2.20E-07	8.17E-07	4.40E-07	4.92E-07	3.01E-07	2.20E-07	5.34E-07	3.46E-07	3.66E-07	1.58E-07
EDI $_{Dermal}$										
Fe	2.62E-08	1.85E-07	1.38E-07	1.16E-07	8.17E-08	2.01E-08	1.47E-07	7.71E-08	8.13E-08	6.34E-08
Cu	1.23E-09	8.48E-09	6.17E-09	5.29E-09	3.70E-09	9.26E-10	7.71E-09	5.09E-09	4.58E-09	3.42E-09
Zn	5.55E-09	1.85E-08	1.11E-08	1.17E-08	6.50E-09	7.41E-09	1.20E-08	1.02E-08	9.87E-09	2.33E-09
Cd	1.08E-10	1.85E-09	1.08E-09	1.01E-09	8.73E-10	7.71E-11	1.23E-09	9.26E-10	7.46E-10	5.99E-10
Cr	1.08E-09	4.01E-09	2.16E-09	2.42E-09	1.48E-09	1.08E-09	2.62E-09	1.70E-09	1.80E-09	7.77E-10
SD = Standard deviation										

Across both seasons, the TR of HM for adults through both ingestion and dermal absorption of the River Nile's water samples consistently were within the "negligible" range for all sampled sites. In the present study, TR values of oral exposure pathway for Cd and Cr ranged from 5.97E-09 to 1.43E-07 and from 1.10E-07 to 4.09E-07, respectively, all classified as negligible. Likewise, the dermal exposure risks for Cd and Cr also were under the negligible rank. Table (6) reveals higher ∑TR from oral exposure compared to dermal adsorption. Moreover, the recorded $\sum TR_{(Oral=Dermal)}$ was consistently below the threshold for any significant cancer risk. Thus, the study indicates that there is no cancerogenic risk from both Cd and Cr through both pathways of exposure, across all sites and seasons studied.

More than 15 million people in the Cairo depend on the River Nile for their daily needs, making it Egypt's main supply of freshwater. A variety of commercial and industrial operations that are essential to the region are also supported by it. But the constant inflow of drainage water from many human activities has been causing significant and harmful alterations to the River Nile. The amount and quality of the river's water are generally affected by various factors, viz. dams, weather patterns, and human activity **(Khalil *et al*., 2017; Salaah *et al*., 2018)**. The largest health hazards were found in water samples from S2, the site of the El-Rahawy drain, although the drain's detrimental effects were also seen at S3, the following location. El-Rahawy drain, identified as a primary contamination source in the River Nile, discharges runoff from agricultural, industrial, and household activities into the river. Hence, S2 receives a wide contaminants' range such as fertilizers, pesticides, and industrial effluents from El-Rahawy drain (**Khalil *et al*., 2017**). Consequently, these pollutants can limit the water usage in this area. Although both the dermal and oral contact with water from the Nile recorded no health hazards at any sampling site, the present study highlights the potential risks associated with using water from S2 and S3 for drinking or irrigation. It is advisable to avoid using this water to prevent potential negative health consequences.

Table 6. Total target cancer risk (TR) f of heavy metals for adults through ingestion and dermal absorption of the Nile water samples throughout the study period

TR_{Oral}						
	Winter			**Summer**		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**
Cd	8.36E-09	1.43E-07	8.36E-08	5.97E-09	9.55E-08	7.161E-08
Cr	1.10E-07	4.09E-07	2.20E-07	1.10E-07	2.67E-07	1.73E-07
Mean	5.91E-08	2.75E-07	1.518E-07	5.79E-08	1.81E-07	1.22E-07
S. D	7.18E-08	1.87E-07	9.64E-08	7.35E-08	1.21E-07	7.15E-08
$\sum TR_{Oral}$	1.18E-07	5.51E-07	3.03E-07	1.15E-07	3.62E-07	2.44E-07
TR_{Dermal}						
Cd	4.10E-11	7.04E-10	4.10E-10	2.93E-11	4.69E-10	3.51E-10
Cr	5.40E-10	2.01E-09	1.08E-09	5.4E-10	1.31E-09	8.48E-10
Mean	2.90E-10	1.35E-09	7.45E-10	2.84E-10	8.90E-10	6.00E-10
S. D	3.52E-10	9.20E-10	4.73E-10	3.61E-10	5.95E-10	3.51E-10
$\sum TR_{Dermal}$	5.69E-10	1.78E-09	1.20E-09	5.69E-10	1.78E-09	1.20E-09
$\sum TR_{(Oral=Dermal)}$	1.18E-07	5.53E-07	3.048E-07	1.16E-07	3.64E-07	2.45E-07
SD = Standard deviation. Cancer risk considered negligible at <10^{-6}: low at <10^{-5}: medium at <10^{-4}: high at <10^{-3} and very high >10^{-3}						

3.2 Ingestion and the Nile tilapia

The assessment of hazard associated with heavy metals for adults *via* normal and habitual consumptions of *O. niloticus* of the River Nile during the study is represented in Tables (7- 9).
Mainly, habitual consumption recorded higher EDI values than normal consumption, while winter recorded higher EDI, compared to summer among all the studied sites. The highest EDI values were detected at site S3, as Fe intake recorded a peak for habitual consumption, followed by Zn> Cu> Cr> Cd during winter and summer. S1 represented the lowest EDI values among sites, while winter revealed higher EDI values compared to summer (Table 7).

Table 7. An inquiry of the evaluated daily intake (EDI) of heavy metals for adults *via* normal and habitual uses of the Nile tilapia

EDI					
Winter				Summer	
		S1	S3	S1	S3
Fe	Normal	2.41E-02	5.55E-02	1.82E-02	4.05E-02
	Habitual	1.10E-01	2.53E-01	8.31E-02	1.85E-01
Zn	Normal	1.11E-02	1.86E-02	1.02E-02	1.53E-02
	Habitual	5.06E-02	8.47E-02	4.67E-02	6.99E-02
Cu	Normal	5.79E-04	1.01E-03	5.44E-04	1.01E-03
	Habitual	2.64E-03	4.60E-03	2.48E-03	4.60E-03
Cd	Normal	5.93E-05	2.96E-04	3.92E-05	2.24E-04
	Habitual	2.71E-04	1.35E-03	1.79E-04	1.02E-03
Cr	Normal	4.90E-04	9.49E-04	4.67E-04	4.78E-04
	Habitual	2.24E-03	4.33E-03	2.13E-03	2.18E-03

In winter, the highest THQ values were recorded at site S3 for Cr, with a value of 7.46E-01 for habitual consumers, followed by Cd> Zn> Fe> Zn. In summer, a different pattern was recorded for habitual consumption, as Cd exhibited the highest THQ values, followed by Cr>Fe> Zn> Cu at S3 (Table 8). The HI in the present study revealed a significant non-carcinogenic risk (HI>1) for habitual consumers associated with consuming *O. niloticus* from S3 during winter and summer (Table 8). Both TR and ∑TR associated with Cd and Cr during summer and winter are demonstrated in Table (9).

Winter recorded the highest TR and ∑TR for habitual consumers at S3, where Cr revealed higher risk than Cd. *O. niloticus* samples from S1 were reported negligible ($TR<10^{6}$) to low ($TR<10^{5}$) carcinogenic risk for both normal and habitual consumers, respectively. While, fish from S3 exhibited medium cancerogenic ($TR<10^{4}$) risks for habitual consumers. Moreover, the ∑TR of *O. niloticus* from S3 recorded high cancerogenic risk ($TR<10^{-3}$) for habitual consumers and medium cancerogenic risk ($TR <10^{-4}$) for normal consumers.

Fish are rich in essential amino acids, minerals, vital polyunsaturated fatty acids, and vitamins that are crucial for human health **(Kawarazuka & Béné, 2011; Reksten *et al*., 2020)**. Fish are a significant rich and affordable source of nutritional animal protein, especially in low- and middle-income countries where hunger and undernutrition are major challenges; these nations count on fish to mitigate these burdens.

Furthermore, food security is an important multifaceted aspect because it is included in many of the Sustainable Development Goals of the United Nations Agenda 2030, including the second objective (Zero Hunger) and the third goal (Good Health and Well-Being) **(WHO, 2019)**. The availability and affordability of fish make it a staple in the Egyptian diet. However, fish collect HM in their tissues at higher ranges than what is allowed, often by multiple times **(Ahmed *et al*., 2022)**. The main method through which humans are exposed to metals is through ingestion. Therefore, consuming seafood that has significant levels of heavy metal contamination can pose several health risks to people, especially for the majority of coastal inhabitants who rely on fish as their main source of animal protein **(Liu *et al*., 2018)**.

Therefore, *O. niloticus* was selected for the present study due to its prevalence in the River Nile and generally, the HMs load in both fish tissues and surface water recorded a relatively high increase in winter to summer. **Hekal and Fahmy (2023)** revealed that during the winter months of December, January, and February, the water flow and levels of the River Nile were the lowest. Conversely, the highest water flow and levels of the River Nile were observed during the summer months of June, July, and August.

Table 8. The target hazard quotients (HQ) and hazard index (HI) of non-carcinogenic health risk of heavy metals for adults via ingestion and dermal absorption of the Nile water samples covering the study period

	THQ *Oral*									
	Winter					Summer				
	Fe	**Cu**	**Zn**	**Cd**	**Cr**	**Fe**	**Cu**	**Zn**	**Cd**	**Cr**
S1	7.63E-06	6.29E-06	6.29E-06	2.20E-05	7.33E-05	5.84E-06	4.71E-06	8.38E-06	1.57E-05	7.33E-05
S2	5.39E-05	4.32E-05	2.10E-05	3.77E-04	2.72E-04	4.27E-05	3.93E-05	1.36E-05	2.51E-04	1.78E-04
S3	4.04E-05	3.14E-05	1.26E-05	2.20E-04	1.47E-04	2.24E-05	2.59E-05	1.15E-05	1.89E-04	1.15E-04
Mean	3.40E-05	2.70E-05	1.33E-05	2.06E-04	1.64E-04	2.36E-05	2.33E-05	1.12E-05	1.52E-04	1.22E-04
SD	2.38E-05	1.89E-05	7.36E-06	1.78E-04	1.01E-04	1.84E-05	1.74E-05	2.64E-06	1.22E-04	5.27E-05
	THQ *Dermal*									
	Winter					**Summer**				
S1	8.74E-08	1.03E-07	9.26E-08	4.32E-06	7.20E-05	6.69E-08	7.71E-08	1.23E-07	3.09E-06	7.20E-05
S2	6.17E-07	7.07E-07	3.09E-07	7.41E-05	2.67E-04	4.89E-07	6.43E-07	2.01E-07	4.94E-05	1.75E-04
S3	4.63E-07	5.14E-07	1.85E-07	4.32E-05	1.44E-04	2.57E-07	4.24E-07	1.70E-07	3.70E-05	1.13E-04
Mean	3.89E-07	4.41E-07	1.95E-07	4.05E-05	1.61E-04	2.71E-07	3.81E-07	1.65E-07	2.98E-05	1.20E-04
SD	2.72E-07	3.09E-07	1.08E-07	3.49E-05	9.88E-05	2.11E-07	2.85E-07	3.88E-08	2.40E-05	5.18E-05

	HI					
	Winter			**Summer**		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**
HI *Oral*	1.16E-04	7.68E-04	4.51E-04	1.08E-04	5.25E-04	3.64E-04
HI *Dermal*	7.66E-05	3.43E-04	1.88E-04	7.54E-05	2.26E-04	1.51E-04
SD = Standard deviation. * HI≤1.0						

According to earlier research by **Rajeshkumar and Li (2018)**, the species, location, and type of tissue were directly influential on the levels of HMs accumulated in fish tissues. The current research findings concur with the heavy metal levels assessed in the study of **Abdel-Halim *et al*. (2022)**. They reported the same seasonal pattern of heavy metal in varying fish species and water off the River Nile, detecting a remarkable HM content increase in water and fish throughout winter, especially for Fe and Zn concentrations. This is the first comprehensive attempt to estimate the potential health risks interrelated with both water usage and consumption of *O. niloticus* from the River Nile.

Basically, the evaluation of human health risks associative with the heavy metal load in edible fish tissues was the key purpose of the present study. The standards of human health estimation are determined by several factors, such as the contaminants ingested, exposure levels and duration, body weight in average, and consumption rates **(Salaah *et al*., 2022)**.

In reference to USEPA hazard guidelines, consuming *O. niloticus* from S3 could induce a non-cancerogenic health risk. While Cr recorded higher lifetime cancer risk for both groups of the Nile tilapia's end- users throughout the study, particularly for habitual consumers. The ΣTR persuaded by consuming *O. niloticus* exhibited higher risk for normal and habitual *O. niloticus* consumers, especially in winter, based on the ΣTR data at S3, consuming *O. niloticus*.

Table 9. A survey illustrating the target cancer risk (TR) and total TR (ΣTR) of heavy metals for adults *via* normal and habitual ingestions of the Nile tilapia in the Nile River

TR			**S1**	**S3**
Cd	**Winter**	**Normal**	2.25E-06	1.12E-04
		Habitual	1.03E-05	5.13E-04
	Summer	**Normal**	1.49E-06	8.50E-05
		Habitual	6.80E-05	3.88E-04
Cr	**Winter**	**Normal**	2.06E-05	3.99E-04
		Habitual	9.40E-05	1.82E-03
	Summer	**Normal**	1.96E-06	2.01E-04
		Habitual	8.95E-05	9.16E-04
∑TR				
Winter		**Normal**	2.28E-06	5.11E-04
		Habitual	1.04E-05	2.33E-03
Summer		**Normal**	2.11E-06	2.86E-04
		Habitual	9.63E-06	1.30E-03
Cancer risk considered negligible at $<10^{-6}$: low at $<10^{-5}$: medium at $<10^{-4}$: high at $<10^{-3}$ and very high $>10^{-3}$				

CONCLUSION

It is important to note that the El-Rahawy drain's effluents, which are influenced by sewage wastewater as well as industrial and agricultural operations, are causing serious environmental degradation in the Rosetta Branch. As demonstrated by the bioaccumulation of heavy metals in its tissues, the results indicated that *O. niloticus* is significantly impacted by this pollution. Because of its resilience to adverse environmental conditions, *O. niloticus* can thus be regarded as a good bioindicator of the contamination caused by heavy metals. The current study emphasizes the possible risks connected with using water from the El-Rahawy drain and El-Qatta area for drinking or irrigation, even though oral and dermal contact with water from the River Nile did not reveal any health problems at any sampling site. This water should not be used in order to avoid any possible health risks. Furthermore, eating *O. niloticus* from the El-Qatta Site carries a significant non-carcinogenic health risk, whereas using Cr increases the risk of developing cancer in the future, especially for regular users, according to USEPA standards. *O. niloticus* consumption in this region carries a high overall cancer risk, which might result in two to five cancer cases per 10,000 normal consumers and one to two cases among thousand habitual consumers over the course of a lifetime. Given the rise in the number of possible cancer cases, this risk was noticeably higher in winter.

These results highlight the disparities in the quality of water across the Nile River and spot the grave consequences of human activity on public health. In order to guarantee safe water use, maintain ecological balance, and protect human health, the current study highlights the need for strong water quality management methods, particularly in areas with greater contamination levels. As a result, the government ought to exert an effort to increase public awareness about the Rosetta Branch. To simultaneously protect both people as end-users and fish from the harmful influences of these contaminants, it is also imperative to exercise greater caution when it comes to the El-Rahawy drain. This includes treating wastewater to remove pollutants before it is released into the Rosetta Branch or establishing regulations that prohibit the discharge of such pollutants into the River Nile.

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