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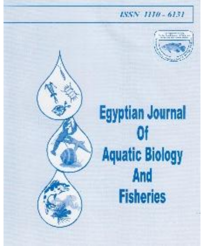

Potential carcinogenic and non-carcinogenic health risks of heavy metals ingestion from consumption of the crayfish, *Procambarus clarkii* in El-Rahawy Drain and El-Kanater in the River Nile, Egypt

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ABSTRACT

Heavy metals can constitute a critical risk to freshwater fauna via exposure, bioaccumulation, and biomagnification processes. The results confirmed that heavy metals in the water, sediments, and tissues of crayfish were considerably higher in El-Rahawy sites in comparison to El-Kanater sites ($P<0.05$). Fe levels were the most abundant concentration compared to the remaining metals (Co, Zn, As, Cd, and Pb) in water, sediment, and crayfish tissues at all sites. Regarding Hms in crayfish tissues, the bioaccumulation sequence of HMs in all sites was in the decreasing sequence of exoskeleton > gills > hepatopancreas > muscles for Co, Pb, and Cd, gills > muscles > Hepatopancreas > Exoskeleton for Zn, and hepatopancreas > gills > exoskeleton > muscles for Fe and As. The HI values of the crayfish muscles, people who eat a normal amount of crayfish have no non-carcinogenic risk. On the other hand, the HI values calculated for habitual crayfish eaters of the muscles crayfish were higher than one which indicates that crayfish muscles have potential non-carcinogenic risk. On contract, As and Cd pose a carcinogenic risk for normal and habitual eaters consuming the muscular crayfish should be considered a hazard message based on data indices and human health viewpoint. Accordingly, the current study recommends a government environmental management in Egypt to conduct routine HM monitoring in the El-Rahawy drain in order to lessen potential health risks.

INTRODUCTION

In Egypt, the water of the River Nile is exposed to several forms of chemical and biological pollutants, additionally with the remains of agricultural wastes and dead animals that are deposited into it. Along the route of the Nile, it gets approximately 37 main drains discharging municipal agricultural and industrial wastewater (**Aboul-Ela *et al.*, 1990**). The primary source of heavy metals in irrigation and drainage canals is the discharge of domestic wastewaters, which contain extremely high concentrations of metals such as copper, iron, lead, and zinc from household products such as cleansing materials, toothpaste, cosmetics, and human feces (**Stephenson, 1987**).

Certain heavy metals, such as zinc, cobalt, copper, manganese, and iron, are required for the growth and survival of living creatures, including humans. They are likely to highlight harmful consequences when the organisms are exposed to amounts beyond the permissible doses. Other variables, such as arsenic, lead, and cadmium, do not seem to be required for metabolic activity and possess hazardous qualities (**EL-Shaikh *et al.*, 2005** and **Ghanem *et al.*, 2015**). Heavy metals are significant contaminants of sediments, water resources, and biota, particularly in industrialized nations, due to their toxicity, persistence, and bioconcentration properties (**Ikem *et al.*, 2003**). The emission of home wastewater with an excessive concentration of metals, such as Al, Cu, Fe, Pb, and Zn is a significant source of heavy metals in irrigation and drainage canals (**APHA**, **1995**).

Crayfish species are important benthic invertebrates, with approximately 640 species spanning three decapod crustacean families (Astacidae, Cambaridae, and Parastacidae) (**Crandall & Buhay, 2008** and **Huner, 2019**). Apart from their ecological importance, they provide an exceptional source of proteins and other necessary elements (**FAO, 2020; Alipour *et al.*, 2021; Schmidt *et al.*, 2021**). *P. clarkii*, a freshwater crawfish, collects heavy metals from the water and sediments in which it dwells. Bioaccumulation of heavy metals in aquatic creatures is a significant phenomenon in ecotoxicology, as several studies have discovered (**Mancinelli *et al.*, 2018** and **Tavoloni *et al.*, 2021**). Consequently, seafood poses potential dangers to consumers, as the primary route of exposure is via the diet. Within the last several decades, potential health hazards associated with heavy metal exposure have been reported. Recent studies have shown that heavy metals provide either carcinogenic or non-carcinogenic dangers to relatives (**Peng *et al.*, 2016** and **Jia *et al.*, 2017**). Heavy metals provide health hazards such as renal failure, bone deformation, and hepatic failure due to their indecomposable and prolonged nature inside the visceral organ parts of people (**Duruibe *et al.*, 2017**). This can result in severe maladies such as dysentery, stomach aches, tremors in the head, anemia, paralysis, nausea, paroxysms, melancholy, and even respiratory disorders (**McCluggage, 1991**), which can manifest in acute or chronic forms; neuron toxicity, oncogenicity, genetic alteration, or teratogenicity (**EU, 2002**). Alternatively, natural products from aquatic organisms were used to sustain human health in ancient times and are currently attractive candidates for multiple human disease therapy and drug discovery **(Cui *et al.*, 2020; Rady and Bashar 2020 and El-Naggar 2022)**.As a result, it is essential to assess the possible dangers to human health associated with the consumption of contaminated food.

The objectives of the present research are to: (1) compare heavy metals concentrations in water, sediment, and tissues of crayfish, *Procambarus clarkii*; (2) establish relationships between heavy metal levels in the environment and, consequently, the crayfish, taking water and sediment characteristics into account and (3) assess the human health risks associated with eating crayfish contaminated with heavy metals. Additionally, it became a goal to validate the possibility of assessing the non-carcinogenic and carcinogenic dangers to human health associated with crayfish muscle ingestion.

MATERIALS AND METHODS

Samples collection

In the present study, sampling of water, sediments, and within the exoskeleton, muscle, gills, and hepatopancreas of crayfish gathered from two sites; the first sites is the El-Kanater sites which are sited at the River Nile and the second one is El-Rahawy drain which drained in the River Nile. Mixed sex crayfish, *P. clarkii*, samples were collected by fishermen during spring-summer, 2021 **(Fig. 1)**. Twenty (20) crayfish individuals were randomly selected from each location. Each sample was properly rinsed with tap water to remove any adherent pollutants and then transferred by icebox to the marine biology laboratory at Al-Azhar University's school of Science. After re-washing thoroughly with potable water, measuring to the closest 1mm, and weighing to the nearest 1 mg, crayfish samples were decapitated, skinned, and dissected (using plastic tools) to extract the exoskeleton, muscles, hepatopancreas, and gills for heavy metals analysis. Simultaneously, water samples were taken from the previously mentioned locations, from the subsurface layer (about 30 cm). A 2L Ruttner Water Sampler container was used to collect samples, which were preserved with 5 ml concentrated nitric acid on the spots and kept in the refrigerator for analysis. Sediment samples were collected from the bottoms of the locations throughout the same time using an Ekman-Grab Sampler (15 x 15cm, 225 cm^2), and were stored in polyethylene bags before being transported to the laboratory for analysis.

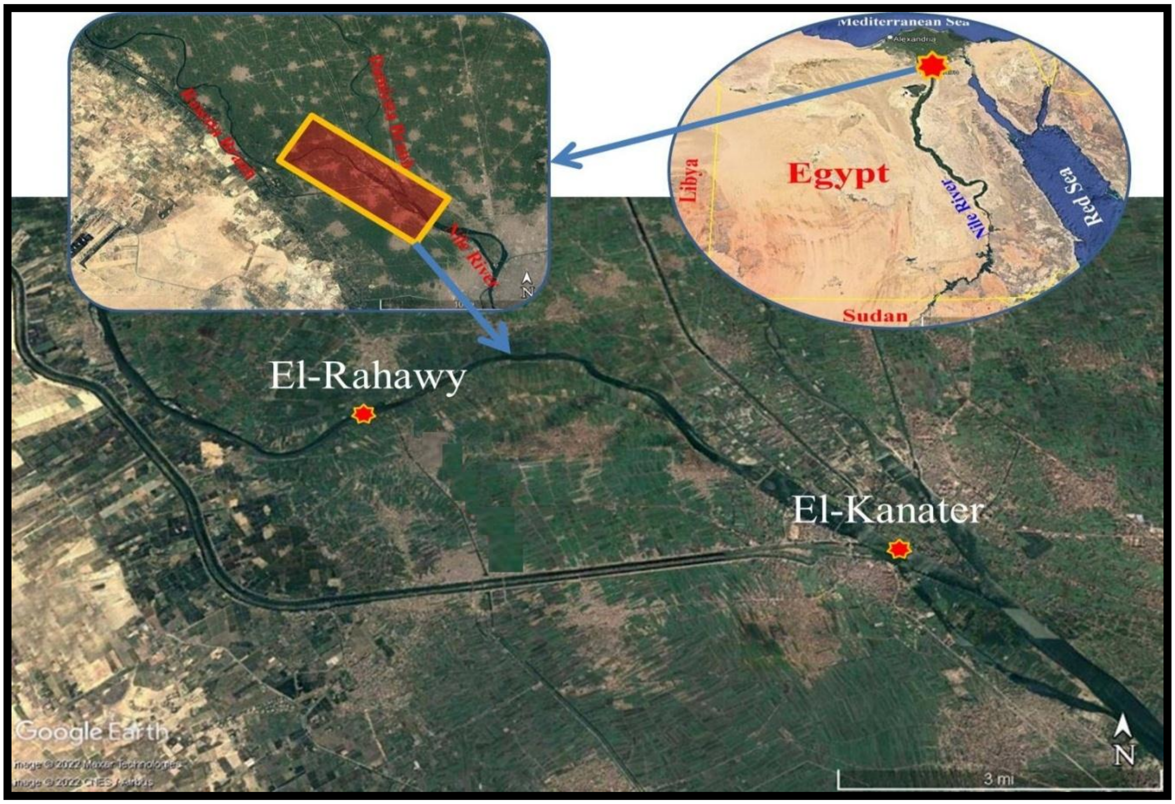

Figure 1. A satellite map of the study area showing El-Kanater sites and El-Rahawy drain.

HMs levels measurement

Following sample collection, HMs analysis was performed on the crayfish examined tissues (exoskeleton, muscles, hepatopancreas, and gills). Each crayfish tissue sample (0.5 g) was put in a 50 mL digestion tube with ultrapure HNO_3 (65%, 2 mL) and H_2O_2 (30%, 1 mL). After 3 hours, samples were digested for 10 hours at 100 °C in an autoclave, cooled to room temperature, transferred to a volumetric flask, and diluted to 50 mL with HNO_3 (1 percent). Five ml concentrated nitric acid was effective for 0.5 g of tissues. The concentrations of HMs in water samples were determined using an acid digestion procedure used to determine the concentrations of total metals. The digested solution was poured into a volumetric flask and diluted to 100 ml with deionized water. Following that, the diluted organ solution and water samples were tested. Sediment samples, on the other hand, were air-dried, crushed, sieved (100 mesh), and dried in a plastic bag. Processed samples (0.5 g) were added to a 50 mL digestion tube together with ultrapure HNO_3 (65%, 5 mL), HF (40%, 2 mL), and $HClO_4$ (40%, 2 mL) (40 percent , 1 mL). The samples were digested for 12 hours at 180 °C in an autoclave. At 140 °C, a heating plate was employed to remove the acid, and the solutions were diluted to 50 mL with 1% HNO_3 in a volumetric flask (**AOAC, 2012**).

The amounts of HMs (cobalt Co, zinc Zn, cadmium Cd, iron Fe, lead Pb, and arsenic as) were evaluated in diluted solutions of organs and water samples. The amounts of HMs in diluted samples (water, sediment, and fish samples, n=5) were determined using an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Model 4300 DV, Perkin Elmer, Shelton, CT, USA). To estimate the ppm of each analyst in the digested solution, samples were treated to a multi-element standard curve. The concentrations of HMs in the water were reported in g/L, whereas those in the sediment and crayfish tissues were expressed in g/g on a dry weight basis.

Health Risk Assessment

After eating crayfish, the human body may collect heavy metals, which is regarded to be a distinct and critical method of exposure (**Ahmed *et al.*, 2019**). The target threat quotient (THQ) was used to assess the non-carcinogenic health risk associated with a combination of the oral reference dose (RfD) and daily intake (EDI), whereas the carcinogenic risk quotient (CRQ) was used to assess the carcinogenic health risk associated with a combination of the oral reference dose (RfD) and daily intake (EDI) (CR).

Human risk assessment calculation

We used the method developed by the U.S. Environmental Protection Agency (**USEPA, 2018b**) to assess the risk to human health of HM consumed by eating the muscles of the investigated fish. The average daily dose established the degree of exposure caused by oral human intake of certain HM observed in fish tissues (Estimated daily intake (**EDI**); average daily intake of a specific chemical over a lifetime). The following equation is used to calculate the **EDI** expressed as mg^{-1} kg^{-1} day^{-1}.

$$\mathbf{EDI = (CF \times IR \text{ x } ER \text{ x } EP / BW \text{ x } AT) \text{ x } 10^{-3}} \qquad \textbf{Mwakalapa et al. (2019).}$$

Where CF equals the average HM concentration in fish muscle - mg/kg wet weight; IR equals the intake rate (0.0312 kg/day for normal consumers and 0.1424 kg/day for habitual consumers); ER equals the exposure rate (365 days/year); EP equals the lifetime exposure period (suspected to be 70 years); BW equals the body weight (suspected to be 70 kg for adults); and AT equals the average lifetime (70 years 365 days.

Evaluating the non-cancer risk

Human risk assessment was performed using the target hazard quotient (THQ), a non-cancer estimate of unfavorable health consequences associated with the consumption of particular HM contaminants found in fish tissues. THQ was determined using the calculation below using the ratio of EDI to ORD (oral reference dose of HMs).

THQ = EDI / ORD (**USEPA, 2018b**).

Where **ORD** represents oral reference doses of HM (mg/kg/days) based on the safe upper limit of HM oral consumption for an adult human weighing 70 kg. Co, Pb, Zn, Cd, As, and Fe have oral **ORDs** of 0.003, 0.00357, 0.3, 0.001, 0.0003, and 0.7 mg/kg/day, respectively (**USEPA, 2018b**). The target hazard quotient (**THQ**) levels below 1.0 suggest negative health consequences for humans are unlikely to occur. In contrast, if the computed **THQ** is larger than 1.0, humans should expect negative health impacts.

The hazard index (**HI**) is additionally, a mathematical formula that reflects the influence of non-carcinogenic hazards by the sum of THQ values of the investigated metals as the following equation:

HI = THQ (Cd)+THQ (Pb)+THQ (Fe)+THQ (Co)+THQ (As)+THQ (Zn) **(USEPA, 2011).**

When the **HI** value is greater than 1, the higher non-carcinogenic risk for the consumers exposed is considered.

Evaluating the cancer risk (CR)

The cumulative risk of acquiring cancer over a lifetime due to heavy metal exposure was described as the incremental likelihood of a person developing cancer multiplied by the cancer slope factor (CSF). The carcinogenic risk may be calculated using the CR value, with CR > $1x10^{-4}$ indicating a high risk of cancer; $1x10^{-4}$ > CR > $1x10^{-6}$ indicating an acceptable risk of cancer; and CR 1 10^{-6} indicating a minimal risk of cancer (**Liang *et al.*, 2018**). The following formula was used to determine the CR:

CR = (ER x EP x EDI x CSF x 10^{-3}) / AT (**Varol *et al.*, 2017**)

where CSF denotes the carcinogenic slope factor, which for As, Cd, and Pb is 1.5, 6.3, and 0.0042 mg kg-1 day-1, respectively (**Wang *et al.*, 2020 and Xiong *et al.*, 2020**).

Statistical analysis

To establish normal distribution and homogeneity of variance, Levene's test was performed. The statistical analyses were conducted using one-way ANOVA (**IBM SPSS Statistical program Version 22; SPSS Inc., IL, USA**), and when statistically significant differences were observed, multivariate, posthoc Tukey assessments were used to calculate the statistical difference between the HMs levels in different crayfish tissues for each metal. The T-test, on the other hand, was employed to compare the crayfish sex groups. The statistics are presented in tables in terms of means and standard division. Statistical significance, however, was established at p 0.05.

RESULTS

HMS concentrations in the water

The concentrations of HMs in the water of the studied area (El-Kanater and El-Rahawy sites) were represented in **Table (1)**. The HMs (Essential heavy metals; Co Fe, and Zn, non-essential heavy metals; AS, Cd & Pb) concentrations in the water of the studied area were significantly decreased in El-Kanater sites compared to El-Rahawy sites ($P<0.05$); being 0.77±0.10 and 4.69±0.57 µg/L, respectively for cobalt, 5.09±1.67 and 31.62±7.37 µg/L, respectively for Zn, 183.73±74.22 and 775.93±115.22 µg/L, respectively for iron, 3.68±0.43 and 22.12±3.36 µg/L, respectively for lead 0.04± 0.01 and 0.10±0.01 µg/L, respectively for arsenic and 0.06±0.01 and 1.78±0.32 µg/L, respectively for cadmium. Iron was the most excessive concentration in the water of El-Kanater and El-Rahawy sites (183.73±74.22 and 775.93±115.22 µg/L, respectively), while arsenic had the lowest values (0.04± 0.01 and 0.10±0.01 µg/L, respectively). HMS levels in the water of the studied sites were in the decreasing sequence in the following order: Fe > Zn > Pb > Co > Cd > As. The permissible limits of different heavy metals in water (µg/L) are shown in **Table 2.**

Table 1. Hm concentrations in the water and sediment collected from El-Kanater and El-Rahawy sites.

	Water samples (mean±SD, µg/L)			**Sediment samples (mean±SD, µg/g dw)**		
	El-Kanater	**El-Rahawy**	Sig.	**El-Kanater**	**El-Rahawy**	Sig.
E-Hms						
Co	0.77±0.10 [b]	4.69±0.57 [a]	0.00001	9.92±1.95 [b]	55.73±9.10 [a]	0.001
Zn	5.09±1.67 [b]	31.62±7.37 [a]	0.004	14.00±0.51 [b]	99.23±11.67 [a]	0.00001
Fe	183.73±74.22 [b]	775.93±115.22 [a]	0.002	53.32±6.04 [b]	123.89±11.56 [a]	0.001
NE-Hms						
Pb	3.68±0.43 [b]	22.12±3.36 [a]	0.001	8.08±0.61 [b]	52.89±6.93 [a]	0.00001
As	0.04± 0.01 [b]	0.10±0.01 [a]	0.104	0.38±0.06 [b]	6.53±0.90 [a]	0.00001
Cd	0.06±0.01 [b]	1.78±0.32 [a]	0.001	0.23±0.02 [b]	1.37±0.39 [a]	0.012

Table 2. Permissible limits of different heavy metals in water (µg/L).

	Heavy metals					
Guidelines	**Co**	**Zn**	**Fe**	**Pb**	**As**	**Cd**
WHO (2011)	50	3000	--	10	3	10
USEPA (2018a)	50	--	--	10	5	15
EC (1998)	--	--	200	10	5	10
EOS (2005)	--	3000	300	10	--	3
CCME (2007) standard	--	50	300	7	--	1
Egyptian guidelines (2013)	--	1000	500	10	10	1

HMS concentrations in the sediment

HMS concentrations in the sediment of the studied area were represented in **Table (1)**. Essential and non-essential heavy metals concentrations in the sediment were significantly decreased in El-Kanater sites in comparison to El-Rahawy drain ($P<0.05$) and it was ordered in the increasing sequence as the following order: Fe (53.32±6.04-123.89±11.56, µg/g DW) > Zn (14.00±0.51-99.23±11.67, µg/g DW) > Co (9.92±1.95-55.73±9.10, µg/g DW) > Pb (8.08±0.61-52.89±6.93, µg/g DW) > As (0.38±0.06-6.53±0.90, µg/g DW) > Cd (0.23±0.02-1.37±0.39, µg/g DW). On a statistical level, analysis of variance (ANOVA, $p<0.01$) was used to analyze data indicating the observed concentrations of heavy metals in water and sediments at the various locations. One-way analysis showed that there are extremely significant variances in all-metal concentrations between the tested locations.

HMS concentrations in crayfish tissues

HMS concentrations in the tissues of crayfish were represented in **Table (3)**. Co Fe, Zn, As, Cd and Pb concentrations in the crayfish tissues from the El-Rahawy drain were significantly higher compared to that collected from El-Kanater ($P<0.05$). Concerning crayfish tissues, HMs in the studied crayfish tissues were in the decreasing sequence of Exoskeleton > Gills > Hepatopancreas > Muscles for Co, Pb, and Cd, Gills > Muscles > Hepatopancreas > Exoskeleton for Zn, and Hepatopancreas > Gills > Exoskeleton > Muscles for Fe and As. Regarding crayfish sex, Co Fe, Zn, and Cd in the tissues of the male crayfish were significantly higher compared to the female samples while Pb and As were significantly lower in the male crayfish compared to the females' samples ($P< 0.05$). Generally, HMs levels in tissues of the crayfish were in the decreasing sequence of Fe (135.4±8.15-709.45±9.12) > Zn (15.19±0.97-99.90±5.22) > Pb (3.14±0.68-49.43±3.46) > As (0.51±0.03-20.07±2.66) > Co (0.86±0.04-9.45±0.36) > Cd (0.50±0.10-5.03±1.61).

Table 3. Heavy metals (µg/g, dw) in the tissues of red swamp crayfish, *Procambarus clarkii* collected from El-Kanater and El-Rahawy sites.

		Muscles		Exoskeleton		Gills		Hepatopancreas	
		Male	Female	Male	Female	Male	Female	Male	Female
Essential HMs									
Co	El-Kanater site	0.92±0.04	0.86±0.04	9.03±0.36	7.30±0.36	3.59±0.14	2.08±0.12	3.13±0.12	1.68±0.14
	El-Rahawy drain	1.17±0.04	1.10±0.04	9.45±0.36	8.47±0.37	4.58±0.14	2.89±0.13	3.92±0.12	2.67±0.14
Zn	El-Kanater site	35.55±1.28	33.66±1.95	19.82±1.17	15.19±0.97	**85.43±3.50**	**46.29±3.38**	28.86±0.93	21.49±0.52
	El-Rahawy drain	39.61±0.57	38.77±0.99	20.41±0.95	18.29±0.75	**99.90±5.22**	**67.45±2.12**	38.82±1.25	30.57±1.99
Fe	El-Kanater site	88.4±9.24	75.4±8.15	**119.08±6.14**	**108.8±6.35**	**382.77±6.93**	**334.07±11.36**	**435.03±8.54**	**394.23±9.01**
	El-Rahawy drain	**121.9±6.39**	**118.01±4.98**	**289.37±6.65**	**226.3±4.95**	**443.5±4.70**	**395.60±5.65**	**709.45±9.12**	**629.65±7.77**
Non-essential HMs									
Pb	El-Kanater site	1.22±0.68	1.74±0.34	24.27±2.58	45.86±4.89	19.78±1.28	38.80±2.50	12.39±0.26	14.93±1.39
	El-Rahawy drain	3.89±0.05	6.00±0.48	37.28±1.50	49.43±3.46	29.85±1.45	45.65±1.40	16.82±1.40	27.27±3.62
As	El-Kanater site	0.35±0.03	0.44±0.15	1.69±0.18	3.19±0.34	3.57±0.23	7.01±0.45	7.40±0.15	10.31±2.19
	El-Rahawy drain	0.70±0.01	1.26±0.09	2.59±0.10	3.43±0.24	5.39±0.26	8.24±0.25	10.05±0.84	20.07±2.66
Cd	El-Kanater site	0.83±0.15	0.50±0.10	7.63±0.84	6.53±1.04	5.03±1.01	3.47±0.99	4.51±1.51	2.95±0.88
	El-Rahawy drain	2.80±1.51	1.24±0.31	9.75±1.31	7.36±1.22	5.03±1.61	3.47±0.31	4.51±1.51	2.95±0.31

Mean±SD with numeric bold is higher than the maximum permissible limit of heavy metals.
Mean±SD without numeric bold is lower than the maximum permissible limit of heavy metals.

According to the results in **Table (4)**, analysis of variance (ANOVA, p0.05) indicates the measured levels of heavy metals in several tissues (exoskeleton, muscle, gills, and hepatopancreas) of red swamp crayfish, *P. clarkii*, collected from various locales (El-Kanater sites and El-Rahawy drain). It was significant differences in the one-way analysis of variance between the different sites, organs, and sex for all metals. However, analysis of two ways of ANOVA provided evidence that there were highly significant differences between different sites and tissues in the same-sex groups for all studied metals, except Cd. On the other hand, the interaction between different sites and sex groups at the same organs was non-significance, except for Co and As which showed significant differences. Moreover, the interaction between different organs and sex groups at the same sites was significant, except for Cd and Fe which showed non-significance differences.

Table 4. Results of analysis of variance (ANOVA) performed on heavy metals recorded from different tissues of red swamp crayfish, Procambarus clarkii collected from El-Kanater and El-Rahawy sites.

Source / Dependent Variable	Sites	Organs	Sex	Sites * organs	Sites *Sex	Organs * Sex
Co	87.256	3038.345	269.237	9.419	7.055	51.873
Zn	194.097	1539.718	444.159	33.474	**0.232**	155.290
Fe	298.841	786.115	82.995	89.913	**0.084**	**1.553**
Pb	111.182	619.359	300.101	8.062	**0.553**	35.413
As	66.438	344.232	115.250	27.659	8.172	26.014
Cd	5.687	81.572	23.996	**1.903**	**1.116**	**0.346**

F- value with numeric bold is non-significant at $p < 0.05$.
F- value without numeric bold is significant at $p < 0.05$.

Human risk assessment

Estimated daily intake (EDI)

Table 5 shows the estimated daily intake (EDI, mg/kg/day) and target hazard quotients (THQ) for trace metals in the muscles of crayfish. EDI in crayfish muscles of El-Rahawy drain ranged from 7E-05 to 2E-02 mg/kg/day for normal consumers and from 3E-04 to 1E-01mg/kg/day for the habitual consumer. In crayfish of El-Kanater sites, however, EDI varied from 5E-05 to 2E-02 mg/kg/day for the normal consumer, and from 2E-04 to 8E-02 mg/kg/day for the habitual consumer.

Table 5. EDI and THQ of heavy metals in red swamp crayfish, *Procambarus clarkii* collected from El-Kanater and El-Rahawy sites.

	El-Kanater				El-Rahawy				
	EDI (mg/kg/day)		THQ		EDI (mg/kg/day)		THQ		
	Normal	Habitual	Normal	Habitual	Normal	Habitual	Normal	Habitual	
Male									**ORDs**
Co	9E-05	4E-04	3E-02	1E-01	1E-04	5E-04	4E-02	2E-01	**0.003**
Zn	4E-03	2E-02	1E-02	6E-02	5E-03	2E-02	2E-02	7E-02	**0.30**
Fe	2E-02	8E-02	3E-02	1E-01	2E-02	1E-01	3E-02	1E-01	**0.04**
Pb	3E-04	1E-03	8E-02	4E-01	4E-04	2E-03	1E-01	5E-01	**0.0036**
As	5E-05	2E-04	2E-02	7E-02	7E-05	3E-04	2E-02	1E-01	**0.003**
Cd	8E-05	4E-04	8E-02	4E-01	3E-04	1E-03	3E-01	**1E+00**	**0.001**
Female									**PTDI**
Co	8E-05	4E-04	3E-02	1E-01	1E-04	5E-04	3E-02	2E-01	--
Zn	3E-03	1E-02	1E-02	5E-02	4E-03	2E-02	1E-02	5E-02	**70**
Fe	1E-02	6E-02	2E-02	8E-02	2E-02	9E-02	3E-02	1E-01	**35**
Pb	5E-04	2E-03	1E-01	6E-01	7E-04	3E-03	2E-01	8E-01	**0.25**
As	9E-05	4E-04	3E-02	1E-01	1E-04	5E-04	4E-02	2E-01	**0.14**
Cd	5E-05	2E-04	5E-02	2E-01	1E-04	5E-04	1E-01	5E-01	**0.07**

- **PTDI**, Permissible tolerable daily intake (mg/day/70 kg, body weight, **FAO/WHO, 2004**).
- **ORDs**, represents for oral reference doses of HMs (mg/kg/days, **USEPA 2018b**).

Non-cancer risk

The **THQ** produced by Hms intake in crayfish and fish muscles is represented in Figs. 3–8. The 95th percentiles of the THQ and HI were used to rank the non-cancer risk of each metal across species and organs.

THQ values calculated for this study were all below 1 implying that consumption of crayfish muscles in the El-Rahawy drain will not impose any health implications for people who consume both normal and habitual amount of crayfish except, Cd was > 1. In crayfish of El-Kanater sites, however, HQ varied from 1E-02 and 1E-01 for the normal consumer, and 5E-02 and 6E-01 for the habitual consumer. Additionally, the order of **HI** in the muscles of a male was HI- El-Rahawy drain > HI- El-Kanater sites, 0.47 and 0.24, respectively for people who eat a normal amount of fish; 2.12 and 1.10, respectively for habitual crayfish eaters. For female crayfish eaters, it was HI- El-Rahawy drain > HI- El-Kanater sites, 0.41 and 0.27, respectively for normal people; 1.87 and 1.22, respectively for habitual crayfish eaters (**Fig. 2**).

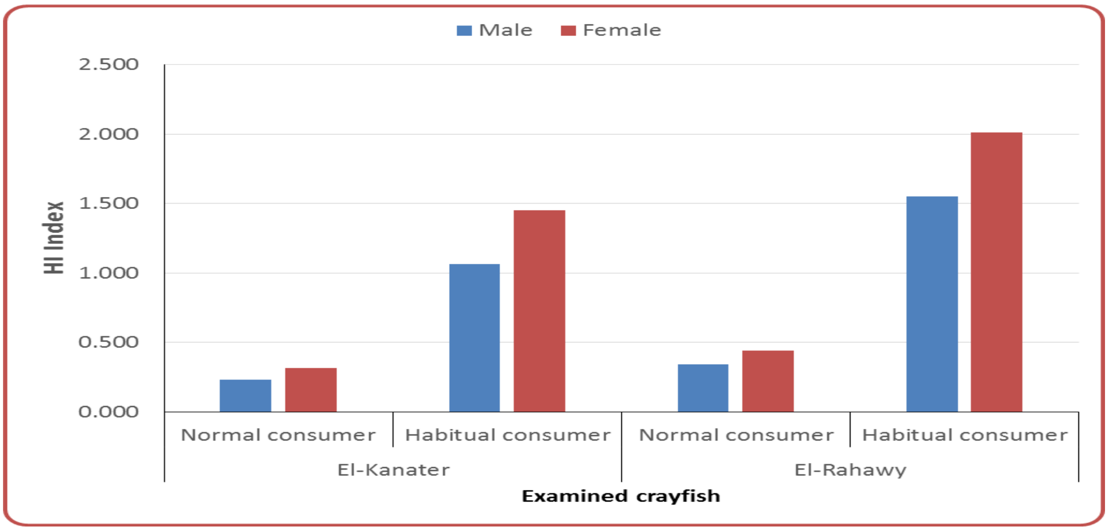

Figure 2. Hazard index (HI) for heavy metals in muscles of red swamp crayfish, *Procambarus clarkii* collected from El-Kanater and El-Rahawy sites.

Cancer risk (CR)

The CR values for As, Cd, and Pb were determined for both normal and habitual consumers in the crayfish muscles of the El-Rahawy drain and El-Kanater sites, and the results are presented in **Figs. (3-5).** The permissible limit for lifelong exposure to carcinogens was determined between 1E–4 (lifetime risk of acquiring cancer is 1 in 10,000) and 1E–6 (risk of developing cancer over a lifetime is 1 in 1,000,000). Using the permissible level of E–4, the CR values for Since and Cd offer a risk of cancer to normal consumers of catfish muscles from both study locations, as the CR values exceeded the specified limit. However, the CR values for Pb in the muscles of crayfish were less than 1E−6 for both normal and habitual consumers.

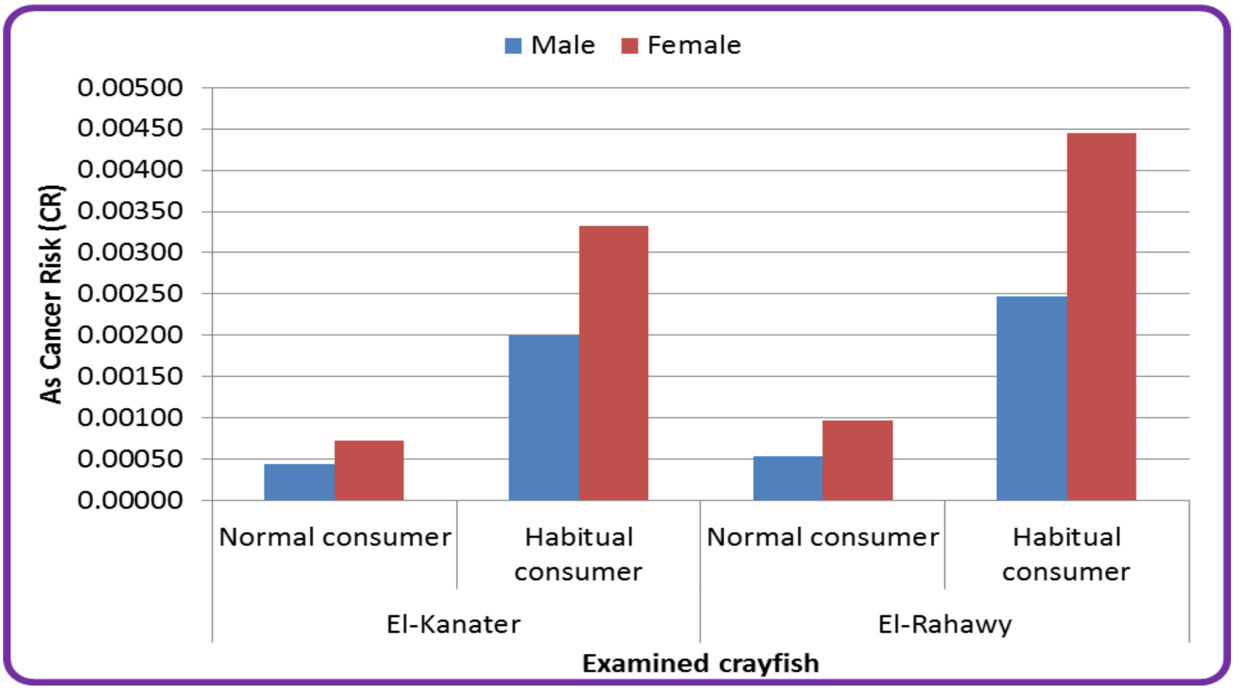

Figure 3. Cancer Risk (CR) of arsenic (As) from consumption of red swamp crayfish, *Procambarus clarkii* collected from El-Kanater and El-Rahawy sites.

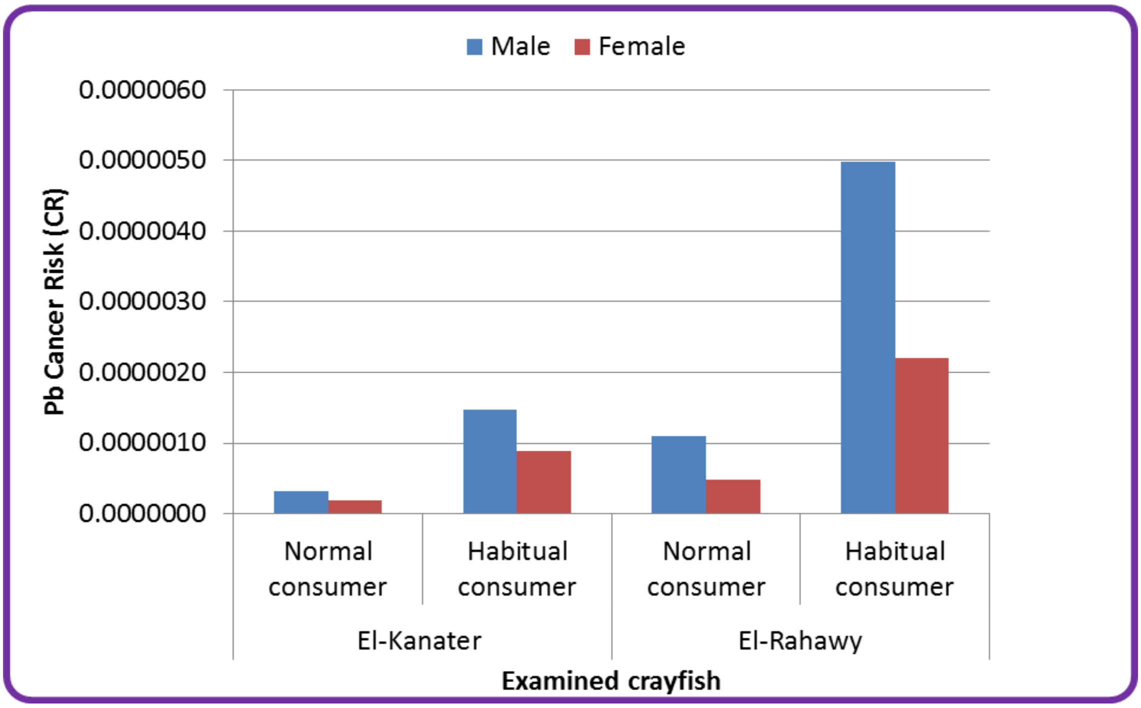

Figure 4. Cancer Risk (CR) of lead (Pb) from consumption of red swamp crayfish, *Procambarus clarkii* collected from El-Kanater and El-Rahawy sites.

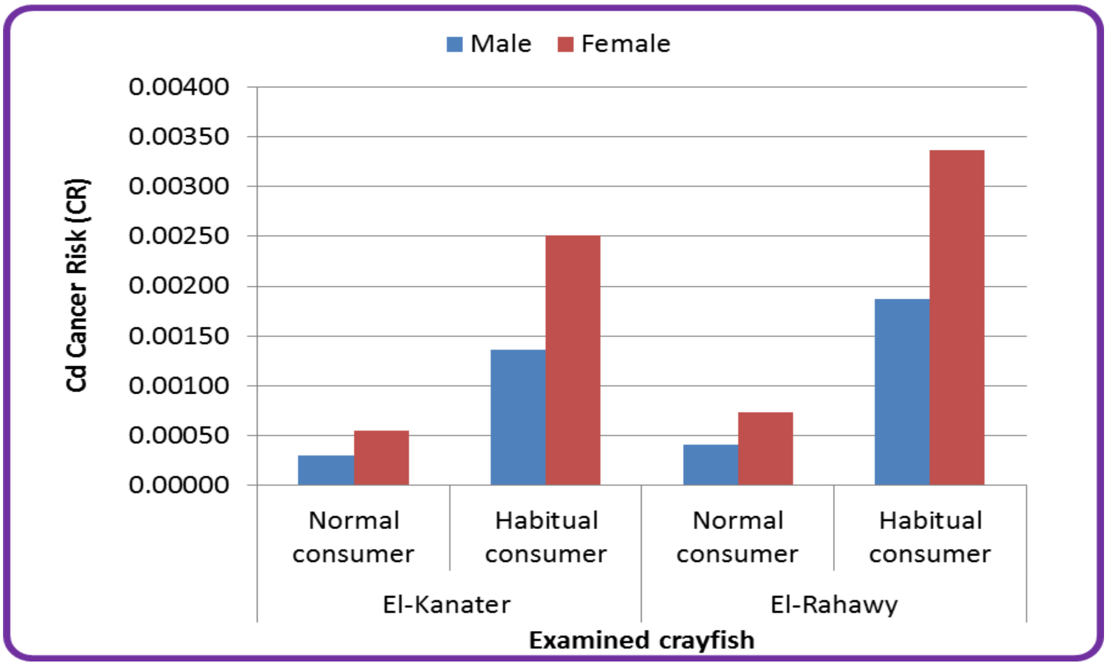

Figure 5. Cancer Risk (CR) of cadmium (Cd) from consumption of red swamp crayfish, *Procambarus clarkii* collected from El-Kanater and El-Rahawy sites.

DISCUSSION

Heavy metals levels in water

Essential heavy metals; Co Fe, and Zn, non-essential heavy metals; AS, Cd & Pb concentrations in the water of the studied area were significantly increased in the El-Rahawy drain compared to El-Kanater sites (P<0.05). These increased concentrations are almost certainly a result of anthropogenic sources (**Abdel-Satar *et al.*, 2017**).

Levels of HMs in the water of the studied sites were in the decreasing sequence in the following order: Fe > Zn > Pb > Co > Cd > As. This arrangement is consistent with Ibrahim and Omar (2013), who said that the order of heavy metals in water samples from locations along the Nile was Fe>Zn>Mn>Cu>Ni>Pb>Cd>Cr>Hg.

Cobalt and arsenic levels in the water of El-Kanater sites and El-Rahawy drain were lower compared to the standard acceptable levels set by **WHO (2011)** and **USEPA, (2018a)**. Also, zinc levels in the water of studied sites were lower compared to the standard acceptable levels set by **EOS (2005), CCME (2007), WHO (2011),** and **Egyptian guidelines (2013)**. Fe and Cd are higher compared to the acceptable limit in the El-Rahawy drain and lower in the El-Kanater sites (**EOS, 2005**; **CCME, 2007; WHO, 2011; Egyptian guidelines, 2013)**. Lead is higher in both sites compared to the standard acceptable levels set by **EOS (2005), CCME (2007), WHO (2011),** and **Egyptian guidelines (2013).** These results were lower compared to those recorded by **El-Bouraie *et al.* (2010)** who reported that Cd and Zn concentrations in the water of the El-Rahawy drain were 2.5 and 32 µg/L, respectively. Also, **El Shakour and Mostafa (2012)** revealed that cadmium, zinc, and iron levels in the water of the El-Rahawy drain were 2, 29, and <200 µg/L, respectively.

Heavy metals levels in sediment

Essential and non-essential heavy metals concentrations within the sediment were significantly decreased in El-Kanater sites compared to the El-Rahawy drain ($P<0.05$). The High levels of all studied metals were recorded at the El-Rahawy drain reflecting the overwhelming influence of wastewater on metals distribution in the Nile water. It had been ordered within the increasing sequence as the following order: Fe > Zn > Co > Pb) > As > Cd. The arrangement of these metals is taken into account in agreement with **Ibrahim and Omar (2013)** who mentioned that the heavy metals in sediment samples from areas of the River Nile were Fe>Zn>Mn>Cu>Ni>Pb>Cd>Cr>Hg.

The cadmium ion in the present study (1.37±0.39 µglg) was higher than that reported by **El-Bouraie *et al*. (2010)** who determined that the Cd in the sediment of the El-Rahawy drain was 0.81 µglg. It was less than 35.68±4.42 µglg observed by Yehia and **Sebaee (2012).** Heavy metals in the studied sediment were lower than that reported by **El Assal and Abdel-Meguid (2017).** Also, **Yehia and Sebaee., 2012** determined the levels of Zn, Fe, and Cd in the sediment of El-Kanater sites were 92.68±0.37, 112.4±8.2, and 30.22±3.82 µglg dw.

Essential metals levels in crayfish tissues

Contamination with heavy metals may be a serious hazard in the aquatic environment. Several of them are critical, serve biological functions in aquatic species, and are referred to as vital heavy metals (**AL-Taee *et al.*, 2020**). Copper, chromium, zinc, nickel, cobalt, and iron are all key metals that the body needs to function well. Otherwise, an insufficient quantity results in deficiency disorders, while an excessive amount results in toxicity (**Sivaperumal *et al.*, 2007 Abadi *et al.*, 2014**). Crayfish absorb hazardous substances, such as heavy metals hundreds or thousands of times greater than levels found in the water, sediment, and diet (**Osman *et al.*, 2007**).

Concerning crayfish tissues, HMs in the studied crayfish tissues were in the decreasing sequence as Muscles > Gills > Hepatopancreas > for Co. However, Co levels of crayfish tissues fluctuated between 0.86±0.04 in muscles and 9.45±0.36 in the exoskeleton. These results are beyond that recorded by **Canpolat *et al.* (2018)** who reported that the level of cobalt in the muscle of crayfish from Turkey ranged between 0.011- 0.052 µg/g with a 0.029 µg/g mean value.

Iron is a critical nutrient for almost all species since it is involved in several components of the photosynthetic apparatus, respiratory processes, DNA synthesis, and hence the delivery and storage of oxygen. Iron may be harmful in high doses, however, the shortage of dissolved iron in the seas is a source of contention **(Galbraith *et al.*, 2019)**. Iron in the studied crayfish tissues was in the decreasing sequence of Hepatopancreas > Gills > Exoskeleton > Muscles. This finding differs from **Abdel Gawad *et al.* (2018)** who recorded that, the highest values of iron levels in the tissues of crayfish collected from the El-Rahawy drain were recorded in the Exoskeleton and the lowest values were observed in muscles.

Zn is a trace element that is present in almost every cell and a broad range of foods. Zn plays a critical function in various metalloenzymes and as a catalyst for controlling the activity of particular Zn-dependent enzymes (**Oehlenschlger, 2002; Marn-Guirao *et al.*, 2008**). Zn is also required for aquatic creatures, such as crayfish; nevertheless, at its maximum concentration, Zn becomes toxic. Numerous experts assume that high Zn levels in marine fish are mostly due to dietary Zn (**Xu and Wang, 2002**). Zinc levels in the studied crayfish tissues were in the decreasing sequence of Gills > Muscles > Hepatopancreas > Exoskeleton. These findings agree with **Gedik *et al.* (2017)** who recorded zinc levels in crayfish tissues were in the decreasing sequence of Gills > Muscles > Hepatopancreas > Exoskeleton and differ with **Abdel Gawad *et al.* (2018)** who mentioned that the maximum value of Zn concentrations indicated was detected in the exoskeleton and the minimum value measured in muscles. Also, **Goretti *et al.* (2016)** observed that the Zn levels higher accumulate in the hepatopancreas (Detoxification tissue) compared to the muscles (Not-detoxification tissue).

Non-essential metals levels in crayfish tissues

Even in low quantities, non-essential heavy metals are considered dangerous (**AL-Taee *et al.*, 2020**). These do not have biological functions and are referred to as xenobiotics; when their concentrations are elevated, they produce homeopathic effects in tissue; these include aluminum, mercury, lead, cadmium, and arsenic, among others (**Sfakianakis *et al.*, 2015**).

Cadmium is considered one of the most toxic heavy metals. It is considered a nonessential element and causes toxic effects on aquatic organisms (**Okocha & Adedeji, 2011; Abbas, 2015**). Pb and Cd in the studied crayfish tissues were in the decreasing sequence of exoskeleton > gills > hepatopancreas > muscles. The current findings indicate that the muscles of the crayfish accumulate the least amount of Cd. These findings corroborated those of the **USEPA (2012),** suggesting that Cd accumulates at low levels in muscle but larger levels in other tissues. Moreover, **Abdel Gawad *et al.* (2018)** revealed that the higher value of Cd and Pb concentrations indicated was detected in the exoskeleton and the lower value was measured in muscles. Moreover, **Goretti *et al.* (2016)** observed that the Cd and Pb concentrations higher accumulate in the hepatopancreas (Detoxification tissue) as compared to the muscles (Not-detoxification tissue).

These results coincide with that mentioned by **Saeed (2011).** This author reported that the gills and liver as highly Pb-accumulated organs in different fish species. **Meyer et al. (1991)** showed that lead levels were elevated in the crayfish *Astacus astacus*, particularly in the digestive gland (hepatopancreas), carapace, and gills, but were extremely low in the hindgut and muscle. These findings disagree with Gedik et al., (2017) who recorded HM levels in crayfish tissues were in the decreasing sequence of hepatopancreas > gills > muscles > exoskeleton for lead and hepatopancreas > gills > exoskeleton > muscles for cadmium.

The least affected tissues were abdominal muscles (**Madigosky *et al*., 1991).** According to **Gilbert and Avenant-Oldewage (2014),** the liver is the principal detoxification organ. The above results showed that the crayfish *P. clarkii* tend to accumulate Cd and Pb in the exoskeleton and hepatopancreas than in the muscles. This indicates that it may be safe for human consumption.

Arsenic enters the aquatic environment through industrial effluents, such as smelting operations and electric generator effluents, as well as agricultural runoff, while arsenic is ingested by gills or polluted feeds (**Ahmed *et al.*, 2008**). The amounts of arsenic in the crayfish tissues investigated decreased in the following order: Hepatopancreas > Gills > Exoskeleton > Muscles. These findings corroborate those of **Ariano *et al.* (2021),** who showed that the greatest concentrations of As in crayfish tissues were found in the hepatopancreas, whereas the lowest concentrations were found in the muscles.

Generally, HMs levels in tissues of the crayfish were in the decreasing sequence of Fe (135.4±8.15-709.45±9.12) > Zn (15.19±0.97-99.90±5.22) > Pb (3.14±0.68-49.43±3.46) > As (0.51±0.03-20.07±2.66) > Co (0.86±0.04-9.45±0.36) > Cd (0.50±0.10-5.03±1.61). These findings differ with **Ariano *et al.* (2021)** who reported that the HM levels in crayfish tissues were in the decreasing sequence of Zn > Cu > As > Cr > Cd.Pb for muscles and Zn > Cu > As > Cr > Cd > Pb for hepatopancreas.

According to **FAO (2016),** iron levels in different tissues of crayfish from the studied sites were exceeding the permissible levels (100 µg/g), except for muscles of El-Kanater sites. However, Zn metals in crayfish tissues from the studied sites were lower than the maximum permissible levels (40 µg/g), except for gills. On the other hand, non-essential metals levels in crayfish tissues from the studied sites were higher than the FAO permissible value of 0.5 µg/g for As, 0.05 µg/g for Cd, and 2 µg/g for Pb **(FAO, 2016)** except muscles from El-Kanter sites.

Human risk assessment

Estimated daily intake (EDI)

We used estimated daily intakes (EDIs) to calculate both the significant non-carcinogenic risk (THQ) and the carcinogenic risk (CR) associated with the use of aquatic products (**Liu *et al.*, 2018**). Daily exposure to harmful substances via the consumption of foods containing heavy metals was utilized to ensure that the absence of any adverse impact on human health occurred throughout the course of a person's lifetime (**Baki *et al.*, 2018**). The EDI values in crayfish muscles from El-Kanater sites and the El-Rahawy drain were between 5E-05 and 2E-02 mg/kg/day for typical users and between 2E-04 and 1E-01 mg/kg/day for habitual consumers. The EDI readings for the majority of heavy metals were below the FAO/WHO Joint Expert Committee on Food Additives' preliminary tolerable daily intake (PTDI) recommendations (**Anandkumar *et al.*, 2020 Xiong *et al.*, 2020**). These findings suggest that the heavy metals consumed as a consequence of crayfish intake are unlikely to pose significant health hazards.

Non-cancer risk

THQ has a permissible limit of one. When THQ is less than the unit limit, it indicates that the exposure amount is less than the reference dosage; consequently, pollution exposure has no detrimental impact on lifetime consumption (**Yi *et al.*, 2011**).

THQ values calculated for this study were all below 1 implying that consumption of crayfish muscles from El-Kanater is for the normal and habitual consumer. However, the THQ values of Cd of crayfish muscles from the El-Rahawy drain were> 1 indicating that poses health implications for people who consume the habitual amount of crayfish muscles.

The **HI** values of the crayfish muscles from the El-Rahawy drain and El-Kanater sites were lower than one for people who eat a normal amount of crayfish. The aforementioned results indicate that crayfish muscles have no non-carcinogenic effects. On the other hand, the **HI** values of the male crayfish muscles from the El-Rahawy drain and El-Kanater sites were 2.12 and 1.10, respectively for habitual crayfish eaters. For female crayfish eaters, it was 1.87 and 1.22, respectively for habitual crayfish eaters. These values are more than one, indicating that crayfish muscles may have anticancer properties in frequent crayfish eaters.

Cancer risk (CR)

As, Cd, Cr, and Pb have been identified as carcinogens by the International Agency for Research on Cancer (**Bonsignore *et al.*, 2018**). The findings indicate that oral intake is the predominant route of exposure to heavy metals. The CR values for As, Cd, and Pb were determined in the crayfish muscles of normal and regular users in the El-Rahawy drain and El-Kanater sites. Using the acceptable limit of E–4, the CR values for Pb in the muscles of crayfish were less than 1E−6 for both normal and habitual consumers. However, As and Cd pose a cancer risk to normal and habitual consumers of crayfish muscles of both studied sites as the CR values were higher than the set limit. These findings show that it is vital to monitor the concentrations of Cd and As metals in crayfish from both locations (El-Rahawy drain and El-Kanater sites) to minimize possible health hazards.

CONCLUSIONS AND RECOMMENDATION

- Heavy metals in water, sediment, and crayfish tissues were significantly higher in El-Rahawy drain compared to El- Kanater station. This indicates that El-Rahawy drain has more heavy metal pollution than El- Kanater station.
- The hepatopancreas of crayfish accumulated higher levels of heavy metals than the other tissues, which is consistent with previous research.
- Muscle of crayfish accumulated heavy metals at the lowest levels when compared to the international (WHO/FAO) fishery products guidelines, the accumulation of metals in the muscle tissues of crayfish was below the maximum permissible limits.
- Heavy metal concentrations in crayfish tissues, particularly the hepatopancreas, appear to be useful pollution biomarkers.
- Finally, As and Cd pose a cancer risk for normal and habitual eaters consuming the muscular crayfish should be considered a hazard message based on data indices and human health viewpoint. Accordingly, the current study recommends a government environmental management in Egypt to conduct routine HM monitoring in the El-Rahawy drain in order to lessen potential health risks.

HIGHLIGHT

- HMs were measured by an inductively coupled plasma optical emission spectrophotometer.
- HMs in the water, sediments, and crayfish were significantly increased in El-Rahawy drain compared to El-Kanater site.
- Hazard index of HM was examined for normal and habitually crayfish eaters.
- As and Cd pose a cancer risk to normal and habitual consumers of crayfish muscles.

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