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Assessment of human risk consumption of fresh water red swamp crayfish, *Procambarus clarkii*

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ABSTRACT

The goal of this research is to determine the concentrations of heavy metals (Copper, Zinc, Lead, and Cadmium) in water and crayfish tissues (Gills, Muscles, and Exoskeleton) collected from three different sites at Fayoum governorate, Egypt. According to the data, the water quality of the three sites differed significantly in terms of physical and chemical properties. The bioaccumulation of lead and cadmium in crayfish muscles from the three sites show an increase in their concentrations. The nutritional value of crayfish meat samples decreased significantly as a result of the study's findings, and they were no longer fit for human consumption. As a result of water deterioration, the protein and fat content of the crayfish muscles, as well as the concentrations of calcium and phosphorus, all decreased significantly.

Keywords: *Procambarus clarkii*, Heavy metals bioaccumulation, Meat quality, human consumption, crayfish muscles.

INTRODUCTION

Heavy metals are a serious problem since they are poisonous and tend to accumulate in vital organs

(Vilizzi *et al*., 2016). Dissolved metals from nearby waters and sediments can be consumed by marine species, accumulating substantial levels in various tissues and causing toxicological effects **(Vilizzi *et al*., 2016)**. Bio-studies can provide us with knowledge about the state of the ecosystem right now **(Zhou *et al*., 2008)**. It can be done by determining the concentration of accumulated metals in an organism's tissues. Aquatic invertebrates are the most effective species to be used as an indicator for an environmental status **(Bonada *et al*., 2006)**. *Procambarus clarkii* **(Girard,1852)** is an invasive organism that originated in the United States and has since spread throughout the world **(Barbaresi and Gherardi, 2000)**. It occupies all types of freshwater ecosystems **(Yi *et al*., 2018)**. Crayfish species are considered ideal bio-indicators of water quality because they have several characteristics, including a high tolerance to water toxins, a large size that makes them suitable for collection and study, and they have a long life cycle **(Gedik *et al*., 2017)**. A wide range of physiological implications, from vital to lethal, result from the rapid mixing of pollutants within the water and their subsequent ingestion by aquatic species. Since fauna can absorb harmful wastes, this one has been widely employed as a key for an environmental monitor to study the impact of contaminants on aquatic life **(El-Moselhy *et al*., 2014)**. Unlike organic molecules, a considerable percentage of metals cannot be efficiently metabolized into less dangerous contaminants. Pollutants are disseminated in the surrounding water, adsorbed in sediments, or eaten by fauna once incorporated into the aquatic ecosystem **(Frémion *et al*., 2016)**. Diverse metal ion builds up has a detrimental influence on aquatic animals' growth **(Atic *et al*., 2010; Bere *et al*., 2012)**. Metallic compounds can affect the amount of oxygen available to crustaceans, as well as their growth and reproductive processes. Certain degenerative alterations occur in fish and crustaceans, such as gill ulceration or liver fibrosis malfunction **(Brraich *et al*., 2015; Sevcikova *et al.*, 2016).** The principal purpose of pollutant accumulation of waste matters in biota or silt is to determine the quality of water and food safety and also to agree with the recommendations. For several factors, the transmission of metallic ions into the aquatic food web may be another interesting point as the concentration of contaminants in aquatic organisms which could be transmitted to humans resulting in a possible risk to community health via the consumption of contaminated food **(Baby *et al*., 2011; Saha *et al*., 2013)**. So the present study aims to follow potential health risk assessment of some heavy metals concentrations via consumption of red swamp crayfish (*Procambarus clarkii*) from river Nile branches at Fayoum governorate, Egypt.

MATERIAL AND METHODS

Sites of collection

Fayoum is a basin located west of the Nile. This basin is watered by a channel of the Nile, the Bahr Youssef. This channel is branched into several canals to cover the Fayoum governorate. Sites were chosen according to the level of pollution caused by human activity such as agricultural runoff and domestic wastes, which are Tanala, Elaam, and El Kaaby at Fayoum governorate, Egypt. Samples of crayfish, *Procambarus clarkii*, and water samples were gathered from three different sites during the period from June to September 2020.

Water samples

Water samples were collected from the three studied sites at a depth of 30 cm below the water's surface (one liter from each site in sterilized and acidified polyethylene bottles). To stop any microbial activity, 5 mL of concentrated HCL was added, then the mixture was placed in an icebox until it was returned to the laboratory. The plastic bottles could be kept at 40 degrees Celsius for a maximum of 24 hours before being analyzed. We followed the guidelines of the American Public Health Association for water and wastewater analysis **(APHA, 2012)**.

Estimation of physicochemical parameters of water samples

Hydrogen ion concentration:

After calibration with buffer solutions during the sampling time, a portable pH meter (Micro Check it ® pH+ Lovibond England) was used to determine the pH of water samples.

Dissolved oxygen (DO)

Water dissolved oxygen of the studied sites of collection, was measured in the field using checkmate II multi-parameter meter.

Nitrite (NO2)

We used a spectrophotometer at wavelength 320 cm^{-1} to quantify nitrite, as described by **George *et al.* (2007)**. We created three tubes of blank, standard and sample tubes, then took readings from each of them. (Reading of Sample tube ÷ reading of Blank tube) × factor

Conductivity

It was measured by using a conductivity meter (PCE-PH 30).

Estimation of heavy metals in water samples

Polyethylene bottles were used to collect samples from the three aquatic different sites, which were then maintained in an icebox until they arrived to the laboratory. Upon arrival to the laboratory, 20.00 ml nitric acid and 5.00 ml H_2SO_4 were added to 500.0 ml of the sample in the beaker at a 4:1 ratio and heated until the lowest volume (approximately 10 ml) was reached, indicating complete digestion, then added deionized distilled water to the remaining until the total volume become 50 ml. Our metals (Cd, Cu, Pb and Zn) were measured using spectrophotometric apparatus, atomic absorption spectrophotometer (SHIMADZU AA-7000) in accordance with the American Public Health Association **(APHA, 2005).** The results are given in parts per million (mg/l). Metal concentration (mg/l) = A x B / C.

Where A = concentration read of atomic absorption, B = volume of diluted solution, C = volume of water sample

Crayfish samples

Samples of crayfish were gathered from the same three studied sites. crayfish samples were cleaned with tap water, then distilled water to eliminate any pollutants, then dissected to take exoskeleton, gills and muscles, then oven-dried at 105 ^{0}c until a constant weight.

Estimation of heavy metal bioaccumulation in crayfish tissues

2g of crushed crayfish tissues were digested with 8 ml of concentrated nitric acid and placed on a heated plate at 70.0 ^{0}c until NO_2 vapor appeared **(Chernoff, 1975)** Then 8 ml of 10% hydrogen peroxide add in the same volume as the previous reagent and heat until the solution turns clear, then cool to room temperature. The filtration process was processed, and the filtrate was put into a volumetric flask and filled to a capacity of 50 ml with distilled water. The solutions were maintained in dark glass bottles at 4° C until analysis, and atomic absorption spectroscopy (SHIMADZU AA-7000) was used to determine the concentrations of the heavy metals investigated (copper, zinc, lead and cadmium). Metal concentrations were declared as mg/kg dry weight of tissue. Metal concentration = A X B / C. Where A= concentration read of atomic absorption, B = volume of diluted solution, C = weight of tissue sample

Estimation of accumulation factor (AF) of a metal ion in muscle samples:

Factor is calculated as the ratio of the pollutant concentration in the specified tissue to the pollutant concentration in the surrounding environment **(Authman and Abbas, 2007).**

Assessment of human risk consumption of edible tissue:

According to the **USEPA (2009)**, the hazard index (HI) was derived using the following equation to measure the risk of human consumption: HI = MDD / ORD

Where MDD (Mean daily dose mg/kg/day) = MC x IRF / NBW

ORD = Oral reference dose of metal ion

MC = Mean concentration of a metal ion in edible tissue expressed by mg /kg dry weight

IRF = Ingestion rate factor = 0.0314 mg /kg / day

NBW= Normal body weight of human = 70 kg, HI is preferred to be < or = 1.

Estimation of crayfish meat quality.

Moisture content:

Carcass moisture content was determined according to **Sidwell *et al*. (1970).**

% moisture content = (muscle weight before dried (g) – muscle weight after dried (g) / muscle weight before dried (g)) X 100

Ash content:

Ash was detected by burning 2.0 g of crayfish muscle in a muffle furnace at 600º C for 16 hrs then cooled and weighed according to **Sidwell *et al*. (1970)**. Ash % = (residual wt. (g) / sample wt. (g)) X 100

Muscles total protein content:

It was determined using the Kjeldahl method, which was developed by **Josyln (1950)**.

Muscles total lipid content:

It was determined using the Soxhlet apparatus according to the standard method documented in AOAC (1980).

Muscles calcium and phosphorus content

The digested solution of the muscle was measured by the spectrophotometric method to assess calcium and phosphorus in crayfish muscles.

Statistical analyses:

The results were expressed as mean ± standard error using statistical processor system support, SPSS software (version 16), IBM, Chicago, IL, USA. Analyses of variance (F- test) and Duncan's multiple ranges were used to examine the homogeneity among the studied sites of collection.

RESULT AND DISCUSSION

Aquatic sites of collection were chosen based on their various levels of contamination at the Fayoum governorate, Egypt notably Tanala, Elaam and El Kaaby. Results revealed that the water quality varied significantly in terms of physical and chemical properties among the studied sites. Usage of invertebrate species as a biological indicator of environmental state is critical for monitoring, evaluating, and combating pollution.

Water Physico-chemical parameters

Hydrogen Ion Concentration (pH)

The pH values of the analyzed aquatic different sites were somewhat alkaline, with the lowest mean value of pH (8.33 ± 0.01) reported at Tanala and the highest mean value of pH (8.53 ± 0.1) recorded at Elkaaby. In addition, the mean pH values in the various aquatic sites studied, were in the following order: Elkaaby > Elaam > Tanala. Table (1) shows the outcomes, the pH values of the Tanala and Elkaaby sites analyzed differed significantly ($P \leq 0.05$) from those of the Elaam site, whereas the last site studied revealed no significant differences from the others. The pH value permitted limits under Egyptian Environmental Law 48 are (6.5-9.0), hence all of the reported ranges in this investigation are within permissible limits.

Dissolved Oxygen (DO)

Table (1) Showed variation of DO of water samples collected from the different studied aquatic sites that revealed a highly significant differences ($P \leq 0.001$). The lowest water dissolved oxygen value was recorded in Elkaaby (3.5 ± 0.03 mg/l), while the highest mean value was reported in Tanala (4.6 ± 0.03 mg/l). The mean DO levels in several aquatic environments were in the following order: Tanala > Elaam > Elkaaby.

Nitrite (NO_2).

The concentrations of nitrite in water samples collected from different aquatic sites represented in table (1). Statistical analyses revealed a significant difference in nitrite values of water samples collected from the different studied aquatic habitats. The highest mean value (0.81 ± 0.003 mg/l) was recorded in Elkaaby and the lowest one was (0.55 ± 0.003 mg/l) in Tanala. The mean values of nitrite at different aquatic habitats followed the order Elkaaby > Elaam > Tanala. Permissible limits for Nitrite is NO_2 (mg/l) < 0.2 by **OATA, 2008**, water nitrite values recorded in the studied sites exceeded the permissible limit.

Conductivity

The values of conductivity for water samples collected from different aquatic sites represented in table (1). Highly significant variations were revealed by statistical analyses ($P \leq 0.001$) in conductivity values of water samples collected from the three studied sites. The highest mean value (1435 ± 1.96 mg/l) was recorded in Elkaaby and the lowest one was (456 ± 2.02 mg/l) in Tanala. The mean values of conductivity at different aquatic habitats followed the order Elkaaby > Elaam > Tanala. Permissible limits for conductivity is not available.

Concentration of heavy metals in water samples:

Concentrations of these metals Cd, Cu, Pb and Zn collected from different aquatic sites were presented in table (1).

Cadmium (Cd)

The maximum mean value of Cd was (7.5 ± 0.001 mg/l) recorded in the Elaam and the lowest one was (0.96 ± 0.01 mg/l) in Elkaaby. In the three sites analyzed an extremely significant difference ($P \leq 0.001$) was found. Permissible limits by WHO (1993) ($Cd \leq 0.01$) so; Cd concentration in the three studied sites exceeded the permissible limit.

Copper (Cu).

The water copper concentration findings showed extremely important variations ($P \leq 0.01$) in the three ecosystems tested. The maximum mean value of Cu was (2.6 ± 0.13 mg/l) found in Elkaaby and minimum mean value was (0.11 ± 0.008 mg/l) recorded in Tanala. The trend of the mean values of Cu in the studied sites of collection followed the order: Elkaaby > Elaam > Tanala. These results are clearly demonstrated in table (1). Permissible limits by WHO (1993) for copper in water (mg/l) ($Cu \leq 1$); the results of water at Elkaaby and Elaam exceeds the permissible limits while Tanala is within permissible limits.

Lead (Pb).

There was a variation in Pb concentration in water samples collected from different studied aquatic habitats. Extremely important variations were observed in the findings at ($P \leq 0.001$) with a maximum mean value of Pb concentration (19.35 ± 0.04 mg/l) in Tanala site of collection while minimum mean value of Pb concentration in Elkaaby was (3.11 ± 0.11 mg/l) as in table (1). Permissible limits by WHO (1993) for lead in water (mg/l) is ($Pb \leq 0.05$). The present results revealed that lead concentration at all three studied sites exceeded the permissible limit.

Zinc (Zn).

Table (1) Showed that the maximum mean value of Zn was (17.42 ± 0.02 mg/l) found in Elaam and minimum one was (3.03 ± 0.2 mg/l) found at Tanala. The mean values of Zn in the studied sites followed the order Elaam > Elkaaby > Tanala. The findings also revealed a very significant difference between the three sites analyzed ($P \leq 0.001$). Permissible limits by WHO (1993) for zinc in water (mg/l) is ($Zn \leq 5$). The result of Tanala is within permissible limits while the results of Elkaaby and Elaam exceeds the permissible limits for zinc ion.

Residual heavy metal bioaccumulation in crayfish muscles:

The mean concentrations of bio accumulated heavy metals Cd, Cu, Pb and Zn in muscle of *Procambarus clarkii* collected from the different studied aquatic sites.

Cadmium (Cd)

The findings showed a highly significant difference (P≤ 0.001) in Cd Concentration *P. clarkii* muscles, collected from Elkaaby and the other two studied sites. Moreover, the maximum mean value of Cd concentration (7.68 ± 0.1 mg/kg dry wt.) that was recorded in muscle of crayfish category from Elkaaby while minimum concentration of Cd (2.28 ± 0.1 mg/kg dry wt.) was recorded in Tanala crayfish, Table (5). Furthermore, the trend of cadmium bioaccumulation in the crayfish muscle followed the order: Elkaaby > Elaam > Tanala. WHO (1993) limits for cadmium in muscle (mg/Kg): Cd (0.5); all the recorded ranges in the present study exceeded WHO limits.

Copper (Cu):

A highly important variation was found in the findings of this research (P≤ 0.001) in Cu concentration values bio accumulated in crayfish muscles collected from the three studied sites of collection (Elkaaby, Elaam, and Tanala). Moreover, the maximum mean value of Cu concentration (16.79 ± 0.3 mg/kg dry wt.) was recorded in muscles of crayfish collected from Elaam while minimum concentration of Cu (0.29 ± 0.02 mg/kg dry wt.) was detected in that of crayfish muscles of Tanala. These results are shown in table (3). Furthermore trend of the bioaccumulation of Cu in the muscles followed the order: Elaam > Elkaaby > Tanala. WHO (1993) limits for copper bioaccumulation in muscle (mg/Kg): Cu (30); all the recorded ranges here are below WHO limits.

Lead (Pb):

Data from this analysis indicated a very significant difference (P≤ 0.001) in Pb concentration values bio accumulated in crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of Pb concentration (23.7± 0.07 mg/kg dry wt.) was recorded in muscles of crayfish from Elkaaby while minimum concentration of Pb (19.2 ± 0.2 mg/kg dry wt.) was recorded in crayfish collected from Tanala. These data are present in table (4). Furthermore, trend of the accumulation of Pb in the muscle followed the order: Elkaaby > Elaam > Tanala. WHO (1993) limits in muscle (mg/Kg): Pb (0.5); all the ranges here exceeded WHO limits.

Zinc (Zn)

There was a significant difference (P≤ 0.001) in the concentration of Zn in *P. clarkii* muscle obtained from three sites tested. Moreover, the maximum mean value of Zn concentration (71.7 ± 0.1mg/kg dry wt.) was recorded in muscle of crayfish category from Elkaaby while minimum concentration of Zn (3.1 ± 0.1 mg/kg dry wt.) was recorded in the Tanala. These data are represented in table (2). Furthermore, trend of the accumulation of Zn in the muscle followed the order: Elkaaby >Elaam > Tanala. WHO (1993) limits for zinc in muscles (mg/Kg): Zn (40); Elaam and Elkaaby results exceeded the permissible limits while Tanala results are within WHO limits.

Heavy metal bioaccumulation in crayfish gills

Zinc (Zn)

A significant variation was observed (P≤ 0.001) in the concentration of Zn in *P. clarkii* gills obtained from three sites examined. Moreover, the maximum mean value of Zn concentration (70.5 ± 0.1mg/kg dry wt.) was recorded in gills of crayfish category from Elkaaby while minimum concentration of Zn (4.1 ± 0.1mg/kg dry wt.) was recorded in the Tanala. These data were represented in table (2). Furthermore, trend of the accumulation of Zn in the muscle followed the order: Elkaaby >Elaam > Tanala.

Cadmium (Cd)

The findings showed a highly significant difference in Cd concentration in *P. clarkii* gills obtained from Elkaaby and the other two sites tested (P≤ 0.001). Moreover, the maximum mean value of Cd concentration (10.9 ± 0.1mg/kg dry wt.) that was recorded in gills of crayfish category from Elkaaby while minimum concentration of Cd (2.39 ± 0.1 mg/kg dry wt.) was recorded in Tanala crayfish (Table (5)). Furthermore trend of cadmium bioaccumulation in the crayfish gills followed the order: Elkaaby > Elaam > Tanala.

Copper (Cu):

The findings of this results shows that there were a highly variations (P≤ 0.001) in the Cu concentration values bio accumulated in crayfish gills obtained from the three sampling sites tested (Elkaaby, Elaam, and Tanala). Moreover, the maximum mean value of Cu concentration (63.4 ± 0.3mg/kg dry wt.) was recorded in gills of crayfish collected from Elaam while minimum concentration of Cu (5.66 ± 0.1 mg/kg dry wt.) was detected in that of crayfish gills of Tanala. These results are shown in table (3). Furthermore, trend of the bioaccumulation of Cu in the gills followed the order: Elaam > Elkaaby > Tanala.

Lead (Pb)

Results from this analysis revealed highly significant variations (P≤ 0.001) in bio accumulated Pb concentration values in crayfish gills obtained from the three sampling sites tested. Moreover, the maximum mean value of Pb concentration (15.3 ± 0.09 mg/kg dry wt.) was recorded in gills of crayfish from Tanala while minimum concentration of Pb (5.79 ± 0.04 mg/kg dry wt.) was recorded in crayfish collected from Elkaaby. These data are present in table (4). Furthermore, trend of the accumulation of Pb in the muscle followed the order Tanala > Elaam > Elkaaby.

Heavy metal bioaccumulation in crayfish exoskeletons

Zinc (Zn)

There was a significant difference (P≤ 0.001) in Zn concentration in exoskeleton of *P. clarkii* collected from three studied sites. Moreover, the maximum mean value of Zn concentration (34.9 ± 0.3mg/kg dry wt.) was recorded in exoskeleton of crayfish category from Elkaaby while minimum concentration of Zn (3.9 ± 0.2 mg/kg dry wt.) was recorded in the Tanala. These data are represented in table (2). Furthermore, trend of the accumulation of Zn in the muscle followed the order: Elkaaby >Elaam > Tanala.

Cadmium (Cd)

Results revealed a highly significant difference (P≤ 0.001) in Cd concentration in exoskeleton of *P. clarkii* collected from Elkaaby and the other two studied sites. Moreover, the maximum mean value of Cd concentration (10.8 ± 0.1mg/kg dry wt.) that was recorded in exoskeleton of crayfish category from Elkaaby while minimum concentration of Cd (0.72 ± 0.1mg/kg dry wt.) was recorded in Elaam crayfish (Table (5)). Furthermore, trend of cadmium bioaccumulation in the crayfish gills followed the order: Elkaaby > Tanala > Elaam.

Copper (Cu):

Results of this study revealed a highly significant differences (P≤ 0.001) in Cu concentration bio accumulated in crayfish exoskeleton collected from the three studied sites of collection (Elkaaby Elaam and Tanala). Moreover, the maximum mean value of Cu concentration (11.6 ± 0.1mg/kg dry wt.) was recorded in exoskeleton of crayfish collected from Elaam while minimum concentration of Cu (0.7 ± 0.001 mg/kg dry wt.) was detected in that of crayfish gills of Elkaaby. These results are shown in table (3). Furthermore, trend of the bioaccumulation of Cu in the gills followed the order: Elaam > Tanala > Elkaaby.

Lead (Pb):

Results from this analysis revealed a highly significant difference (P≤ 0.001) in bio accumulated Pb concentration values in exoskeleton crayfish obtained from the three sampling sites tested. Moreover, the maximum mean value of Pb concentration (26.94 ± 0.1mg/kg dry wt.) was recorded in exoskeleton of crayfish from Elkaaby while minimum concentration of Pb (3.04 ± 0.1mg/kg dry wt.) was recorded in crayfish collected from Elaam. These data are present in table (4). Furthermore, trend of the accumulation of Pb in the muscle followed the order Elkaaby >Tanala >Elaam.

Accumulation Factor (AF) of copper in crayfish muscles

Results from this analysis revealed highly significant variations (P≤ 0.001) in the bio accumulated Cu-AF concentration values in crayfish muscles obtained from the three sampling sites tested. At the sites of Tanala and Elaam the AF values indicate a highly statistical difference (P≤ 0.001) whereas at the site of Elkaaby a slight significant difference (P≤ 0.05). Moreover, maximum mean value of Cu- AF concentration (12 ± 0.5 mg/kg dry wt.) was recorded in muscles of crayfish from Elaam while minimum concentration of Cu-AF (2.5 ± 0.1mg/kg dry wt.) was recorded in crayfish collected from Tanala. These data are present in Fig. (1). Furthermore, trend of the accumulation of Cu-AF in the muscle followed the order Elaam > Elkaaby >Tanala.

Accumulation Factor (AF) of Zinc in crayfish muscles

Results from this analysis revealed highly significant differences (P≤ 0.001) in bio accumulated Zn-AF concentration values in crayfish muscles obtained from the three sampling sites tested. Moreover, the maximum mean value of Zn-AF concentration (5.8 ± 0.003 mg/kg dry wt.) was recorded in muscles of crayfish from Elkaaby while minimum concentration of Zn-AF (1.03 ± 0.07 mg/kg dry wt.) was recorded in crayfish collected from Tanala. These data are present in Fig. (2). Furthermore, trend of the accumulation of Cu-AF in the muscle followed the order Elkaaby > Elaam >Tanala.

Accumulation Factor (AF) of lead in crayfish muscles

Data from this analysis indicated a very significant difference (P≤ 0.001) in Pb-AF concentration values bio accumulated in crayfish muscles collected from the three studied sites of collection. The values of Pb- AF show a higher significant difference (P≤ 0.001) at Elkaaby while there is no significant difference in values at the two other sites. Moreover, the maximum mean value of Pb-AF concentration (8.6 ± 0.5 mg/kg dry wt.) was recorded in muscles of crayfish from Elkaaby while minimum concentration of Pb-AF (0.9 ± 0.02 mg/kg dry wt.) was recorded in crayfish collected from Tanala. These data are present in Fig. (3). Furthermore, trend of the accumulation of Pb-AF in the muscle followed the order Elkaaby > Elaam >Tanala.

Accumulation Factor (AF) of Cadmium in crayfish muscles

Data from this analysis indicated a very significant difference (P≤ 0.001) in Cd-AF concentration values bio accumulated in crayfish muscles collected from the three studied sites of collection. The values of Cd- AF show a higher significant difference (P≤ 0.001) at Elkaaby while there is no significant difference in values at the two other sites. Moreover, the maximum mean value of Cd-AF concentration (10.7 ± 0.1mg/kg dry wt.) was recorded in muscles of crayfish from Elkaaby while minimum concentration of Cd-AF (0.8 ± 0.1mg/kg dry wt.) was recorded in crayfish collected from Elaam. These data are present in Fig. (4). Furthermore, trend of the accumulation of Cd-AF in the muscle followed the order Elkaaby > Tanala >Elaam.

Meat quality of the studied fresh water crayfish, *Procambarus clarkii*

Moisture content:

Data from this analysis indicated a very significant difference (P≤ 0.001) in the values of moisture content of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of moisture content of crayfish muscles (75 ±0.1%) was recorded in muscles of crayfish from Elkaaby while minimum moisture content of crayfish muscles (65 ± 0.1 %) was recorded in crayfish collected from Tanala. These data are present in table (6). Furthermore, trend of the moisture content of crayfish muscles followed the order Elkaaby > Elaam >Tanala.

Ash content

Data from this analysis indicated a very significant difference (P≤ 0.001) in the values of ash content of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of ash content of crayfish muscles (15.33 ± 0.1%) was recorded in muscles of crayfish from Elkaaby while minimum ash content of crayfish muscles (10.23 ± 0.1%.) was recorded in crayfish collected from Tanala. These data are present in table (6). Furthermore, trend of the ash content of crayfish muscles followed the order Elkaaby > Elaam >Tanala.

Total protein

Data from this analysis indicated a very significant difference (P≤ 0.001) in the values of total protein content of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of total protein content of crayfish muscles (17 ± 0.01%) was recorded in muscles of crayfish from Tanala while minimum total protein content of crayfish muscles (10 ± 0.01%.) was recorded in crayfish collected from Elkaaby. These data are present in table (6). Furthermore, trend of total protein content of crayfish muscles followed the order Tanala > Elaam >Elkaaby.

Total lipids

Data from this analysis indicated a very significant difference (P≤ 0.001) in the values of total lipid content of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of total lipid content of crayfish muscles (4.52 ± 0.01%) was recorded in muscles of crayfish from Tanala while minimum total lipid content of crayfish muscles (2.52 ±

0.01%.) was recorded in crayfish collected from Elkaaby. These data are presented in table (6). Furthermore, trend of total lipid content of crayfish muscles followed the order Tanala > Elaam >Elkaaby.

Calcium content

Data from this analysis indicated a very significant difference (P≤ 0.001) in total calcium content of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of calcium content of crayfish muscles (70.5 ± 0.1 mg/kg) was recorded in muscles of crayfish from Tanala while minimum calcium content of crayfish muscles (46.25 ± 0.1mg/kg.) was recorded in crayfish collected from Elkaaby. These data are present in table (6). Furthermore, trend of calcium content of crayfish muscles followed the order Tanala > Elaam >Elkaaby.

Phosphorus content

Data from this analysis indicated a very significant difference (P≤ 0.001) in total phosphorus content of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of phosphorus content of crayfish muscles (600 ± 0.02 mg/kg) was recorded in muscles of crayfish from Tanala while minimum phosphorus content of crayfish muscles (530 ± 0.04mg/kg.) was recorded in crayfish collected from Elkaaby. These data are present in table (6). Furthermore, trend of phosphorus content of crayfish muscles followed the order Tanala > Elaam >Elkaaby.

Assessment of human risk consumption of edible crayfish muscles (HI)

Copper- HI

Data from this analysis indicated a highly significant difference (P≤ 0.001) in Cu-HI of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of HI of crayfish muscles (0.167 ± 0.0005 mg/kg) was recorded in muscles of crayfish from Elaam while minimum HI of crayfish muscles (0.003 ± 0.0005 mg/kg.) was recorded in crayfish collected from Tanala. These data are presented in Fig. (5). Furthermore, trend of HI of crayfish muscles followed the order Elaam > Elkaaby >Tanala.

Zinc - HI

Data from this analysis indicated a highly significant difference (P≤ 0.001) in Zn-HI of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of HI of crayfish muscles (0.095 ± 0.001 mg/kg) was recorded in muscles of crayfish from Elkaaby while minimum HI of crayfish muscles (0.004 ± 0.001 mg/kg.) was recorded in crayfish collected from Tanala. These data are presented in Fig. (6). Furthermore, trend of HI of crayfish muscles followed the order Elkaaby > Elaam >Tanala.

Lead- HI

Data from this analysis indicated a highly significant difference (P≤ 0.001) in Pb- HI of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of HI of crayfish muscles (3.16 ± 0.008 mg/kg) was recorded in muscles of crayfish from Elkaaby while minimum HI of crayfish muscles (2.56 ± 0.005 mg/kg.) was recorded in crayfish collected from Tanala. These data are presented in Fig. (7). Furthermore, trend of HI of crayfish muscles followed the order Elkaaby > Elaam >Tanala.

Cadmium HI

Data from this analysis indicated a highly significant difference ($P \leq 0.001$) in Cd- HI of crayfish muscles collected from the three studied sites of collection. Moreover, the maximum mean value of HI of crayfish muscles (4.14 ± 0.005 mg/kg) was recorded in muscles of crayfish from Elkaaby while minimum HI of crayfish muscles (1.12 ± 0.005 mg/kg.) was recorded in crayfish collected from Tanala. These data are presented in Fig. (8). Furthermore, trend of HI of crayfish muscles followed the order Elkaaby > Elaam >Tanala.

Discussion

Environmental pollution has been recorded as the most pressing issue for decades as it harms human wellbeing and the whole surrounding living organisms. Among environmental pollution, heavy metal contamination is one of the most dangerous types of pollution because of its accumulation in biological issues and failure to excrete and it has resulted in several severe health issues around the world. Heavy metal concentrations in the natural environment are measured in water, soil, and living organisms but since the majority of heavy metals concentrations in living organisms and soil, the amount within water has been deemed inadequate to determine environmental toxicity (Lasheen ***et al***., 2012; Rashed, 2001). Red swamp crayfish can ingest toxic metals from food chains (Anandkumar, 2020).

The most important indicator of the biological and chemical status of the ecosystem is the pH value of the aquatic ecosystem (Osman ***et al***., 2010). The ability of bacteria to degrade toxic compounds into a non-toxic form is influenced by their pH value (Adeogun, 2012). The pH of the aquatic sites analyzed was slightly alkaline, this slightly elevated pH value is attributed to a variety of waste sources that have been released into the aquatic environment. The average pH values in studied aquatic sites were Elkaaby >Elaam> Tanala. The oxygen content of water samples collected from the three studied aquatic sites revealed highly significant differences ($P \leq 0.001$) between them. Dissolved oxygen mean values in studied aquatic sites were in the following order: Tanala >Elaam> Elkaaby. As emission levels rise, the oxygen content value decreases, consequently biota become more vulnerable to environmental stressors (Osman ***et al.***, 2010). The increasing nitrite concentration leads to growing algae in large amounts which causes depletion in oxygen content. The findings showed major variations in the nitrite mean values between the three studied aquatic sites, the highest mean value was found in Elkaaby while the lowest ones in Tanala (Elkaaby > Elaam > Tanala). The allowable limit for water nitrite in studied sites was exceeded. While the conductivity means values of water samples collected from three study sites, varied significantly ($P \leq 0.001$), The highest mean value was found in Elkaaby while the lowest ones in Tanala (Elkaaby > Elaam >Tanala). Since the conductivity parameter indicates anthropogenic activity, we can predict that the region with the highest conductivity value (Elkaaby) is the most contaminated one. Heavy metals Zn, Cu, Pb, and Cd were estimated at the three studied aquatic sites, the results showed that the maximum mean value of Zn found at Elaam and minimum one at Tanala (Elaam > Elkaaby > Tanala) with a very significant difference ($P \leq 0.001$) between the three aquatic sites analyzed. Zinc mean values in the water of two aquatic sites (Elaam and Elkaaby) exceeded the permissible limits by WHO (1993) and this may be attributed to effluents of agriculture that rich in fertilizer chemicals. While maximum mean values of water copper concentration were found at the Elkaaby site (Elkaaby > Elaam > Tanala), the results of copper mean values of Elkaaby and Elaam sites exceeded the permissible limits by WHO (1993) while Tanala is within permissible limits. The high concentration of copper in the environment may be due to agricultural wastes and domestic Sewage and this is in agreement with Mahmoud ***et al***. (2005). Lead and cadmium metal ions are xenobiotic metals that can be harmful even in any dose (Badr ***et al.***, 2014). The present results revealed that the lead concentration at all three studied sites exceeded the permissible limit, with extremely significant differences ($P \leq 0.001$) with a maximum mean value of Pb concentration in Tanala and a minimum one in Elkaaby. The rise in lead concentration may be due to pesticides containing lead, lead dust fallout, or street runoff. While cadmium mean values were maximum in Elaam and the lowest one in Elkaaby, Cd concentrations in the three studied sites exceeded the permissible level by WHO (1993).

The concentration of heavy metals (zinc, copper, lead, and cadmium) was studied in some selected crayfish vital organs (gills, exoskeleton, and muscles) of *Procambarus clarkii*, collected from three different studied sites (Tanala, Elaam, and Elkaaby) at Fayoum governorate, Egypt. Some metals accumulate in crustaceans in direct relation to their availability within water (Bryan ***et al.***, 1965) but in our study, we concluded that *Procambarus clarkii* accumulate heavy metals in their tissues irrespective of their concentration in the ambient water and this agrees with Abdel-Meguid (2017). Gills are the primary sites of exposure and uptake of metal ions from the outside atmosphere reflecting the consistency of the external environment (Bryan, 1960). The presence of metal ions in gills can be due to metal adsorption on the surface of the gills. Metal complexation with mucus (contain - SH group) that is difficult to extract from lamellae until examination could inform the high metal amount in the gills (Mohamed ***et al***., 2017). One of the key factors for the increased presence of metal ions in gills is their ability to absorb metals carried in by blood from other parts of the body and stimulate the formation of metallothionein a metal-binding protein that is thought to protect against the toxic effects of heavy metals by binding them (Peters ***et al.***, 1985). Our investigation study shows that the cadmium level in our crayfish tissues is in the following order Gills > Exoskeleton > Muscles, and this is in agreement with Bruno ***et al.***, (2006), Gedik ***et al.***, (2017), Annabi ***et al.,*** (2018), Subotic ***et al.***, (2019) Anandkumar ***et al.***, (2020) and Tan ***et al***., (2021). The low level of metals in muscles could be due to a lack of blood supply and lower metabolic activities of muscle (Mohamed ***et al.***, 2009) whereas the level in the exoskeleton could be due to adsorption on the exoskeleton's surface similar to what Wright discovered (1977). Although the exact mechanism of metal on absorption is unclear. Lead is not essential for living organisms and has many hazardous effects on living organisms (Allert ***et al.***, 2009). Our investigation study shows that the lead level in our crayfish tissues in the following order Muscles > Exoskeleton > Gills and this result in agreement with Mackevičienė, (2002) and Suárez-Serrano ***et al.,*** (2010) and Gedik ***et al.,*** (2017) but this disagrees with Anderson ***et al.*** (1997). Our results indicated that cadmium and lead accumulated in crayfish samples in the following order Elkaaby > Tanala > Elaam and their concentration in muscles exceeded the permissible limits by WHO (1993). Our results indicated that copper accumulated in crayfish samples in the following order Elaam > Elkaaby > Tanala. Its concentration in red swamp crayfish tissues is in the following order Gills > Muscles > Exoskeleton and this is in agreement with Goretti *et al.*, (2016), Annabi *et al.*, (2018b), Anandkumar *et al.*, (2020) Subotić *et al.*, (2019). According to Naqvi *et al.* (1998), the decrease in Cu accumulation in abdominal muscle is due to the deficiency of holding binding molecules in abdominal muscle tissue. While Zinc concentration in crayfish samples in the following order Elkaaby > Elaam >Tanala. Zinc level in our crayfish tissues in the following order Muscles > Gills > Exoskeleton. The high concentration of zinc in our muscle samples than other tissues are in agreement with Naghshbandi *et al.* (2007) and Protasowicki *et al.* (2013) Anandkumar *et al.* (2020) but disagree with Mackeviciene, (2002) who determined a high concentration of zinc ion in exoskeleton this attributed to its role in excretion process of this metal. Trace elements can be ingested by humans via the food chain causing acute and chronic health effects. Aquatic species absorb trace elements in small quantities by absorption from the water column and in greater amounts through trophic transport from prey (Anandkumar *et al.*, 2018a). Humans consume protein omega-3 fatty acids vitamins calcium and other nutrients found in marine products all over the world (Anandkumar *et al.*, 2018b). Abdominal muscle tissue is particularly important and valuable in terms of health since it is the most nutritious portion of the aquatic organism and is commonly consumed by humans. These results suggest that *P. clarkii* has a high capacity for bio accumulating toxic elements and as a result the ability to transfer toxic elements to higher trophic levels. Meanwhile, crayfish are consumed by fish, birds, and humans promoting heavy metal bio magnification through the entire trophic network (Aquino *et al.*, 2017). Because of their central role in the food web crayfish are thought to be a vector for transmitting toxic metals to top predators (including human populations) through tropic transfer. Our results show an increase in lead and cadmium concentration in our crayfish muscles collected from three studied sites. For edible muscles, the values of HI were within safe limits for Zn and Cu in all studied areas. These findings suggest that to be exposed to the health risks associated with Zn and Cu, more crayfish would need to be consumed. Lead and cadmium, on the other hand, had high HI values (>1) and were found to have harmful effects on human health at all of the studied sites. These health troubles are very considerable such as renal failure, liver damage, cancer, and nervous system damage (Malik *et al.*, 2010). This agrees with Khalil *et al.*, (2013) who reported that Pb and Cd exceeded the guidelines and the permissible limits in this species. According to the USEPA, (2012), ingestion of fish with a total Hazard Index (HI) below one presents no health danger while greater than 10 denotes a high risk, and between 1 and 10 represent the moderate state (Ukoha *et al.*, 2014). It can be concluded that consumption of these animals in large quantities for a long time or from contaminated areas could cause adverse health results and this agrees with Khalil *et al.*, (2013).

Meat chemical composition (Ash percent, moisture content, calcium, phosphorus, total protein, and total lipids) of crayfish muscles collected from the three studied aquatic sites, show a highly significant difference ($P \leq 0.001$) between them. Results show that moisture content of crayfish muscles was collected from three studied sites of collection in the following order Elkaaby > Elaam >Tanala, while the trend of the ash content followed the order Elkaaby > Elaam >Tanala. Calcium, phosphorous, Total protein, and lipids contents follow the order Tanala > Elaam > Elkaaby. From these results, we concluded that the meat quality of our crayfish samples is affected by habitat pollution levels. As pollution level increases the quality of crayfish meat decreases and become unsuitable for human consumption this is in agreement with the point of view of Berge *et al.*, (2004) which indicated that Chemical composition and nutritional values of meat quality are affected by environmental conditions. In conclusion, the present work declares that the freshwater red swamp crayfish, *Procambarus clarkii*, can stand as a high source of protein and other main chemical compositions. Meanwhile, the recorded heavy metals bioaccumulation in crayfish vital organs (gills, muscles, and exoskeleton) resemble hazardous risk to humans for consumption. Otherwise, it could be cultured or collected from water resources with high sanitary quality.

Table (1): Physico-chemical parameters (mean ± standard error) of water at three aquatic sites at Fayoum Governorate, Egypt.

	Tanala	Elaam	Elkaaby	permissibl elimits	F value
PH	8.33±0.01*	8.35±0.05	8.53±0.12*	6.5-9	5.6*
DO ppm	4.6±0.03***	4.1± 0.03***	3.5±0.03***	≥5	276***
Conductivity µs	456±2.02***	535±1.71***	1435±1.96***	NA	81495***
(NO2) ppm	0.55±0.003***	0.65±0.003***	0.81±0.003***	< 0.2	1640***
Cu ppm	0.11±0.008***	1.4±0.08***	2.6±0.13***	1.0	960.6***
Zn ppm	3.03±0.20***	17.42±0.02***	12.3± 0.05***	5.0	46836***
Pb ppm	19.35±0.04***	14.88±0.04***	3.11±0.11***	0.05	9297***
Cd ppm	1.91±0.04***	7.5±0.001***	0.96±0.01***	0.01	12107***

Permissible limits according to **WHO (1993)** in cases of Cu, Zn, Pb, Cd while in cases of PH, DO, Conductivity according to **Egyptian Law No.48 (1982),** NA means not available. Permissible limits for Nitrite is (NO_2 (mg/l) < 0.2 by OATA, 2008). Results are presented as means of five samples ± SE, * significant (P<0.05), *** Highly significant (P<0.001).

Table (2): Zinc concentrations in some vital organs of *Procambarus clarkii* (mg/kg dry wt.) collected from three aquatic sites at Fayoum Governorate, Egypt.

Studied sites	Exoskeleton	Gills	Muscles P.l.= 40 ppm
Tanala	3.9±0.2***	4.1±0.1***	3.1±0.1***

Elaam	20.8±0.1***	28.8±0.1***	63.2±0.2***
Elkaaby	34.9±0.3***	70.5±0.1***	71.7±0.1***
F- value	4664***	149970***	287455***

Permissible limits according to **WHO (1993).** Data are presented as means of five samples ±SE, *** Highly significant (P<0.001).

Table (3): Copper concentrations in some tissues of *Procambarus clarkii* (mg/kg dry wt.) collected from three aquatic sites at Fayoum Governorate, Egypt.

Studied sites	Exoskeleton	Gills	Muscles P.l = 30 ppm
Tanala	1.6±0.02***	5.66±0.1***	0.29±0.02***
Elaam	11.6±0.1***	63.4±0.3***	16.79 ±0.3***
Elkaaby	0.7±0.001***	26.5±0.5***	10.40±0.3***
F- value	4652***	4645***	752***

Permissible limits according to **WHO (1993).** The data is described as an average of five samples ± SE, *** Highly significant (P<0.001).

Table (4): Lead concentrations in some *tissues of Procambarus clarkii* tissues (mg/kg dry wt.) collected from three aquatic sites at Fayoum Governorate, Egypt.

Studied sites	Exoskeleton	Gills	Muscles P.l.= 0.5 ppm
Tanala	20.69±0.2***	15.3±0.09**	19.2±0.2***
Elaam	3.04±0.1***	14.7±0.1**	21.5±0.1***
Elkaaby	26.94±0.1***	5.79±0.04***	23.7±0.07***
F- value	3669***	3142***	181***

Permissible limits according to **WHO (1993).** The data is described as an average of five samples ± SE, *** Highly significant (P<0.001).

Table (5): Cadmium concentrations in some tissues of *Procambarus clarkii* (mg/kg dry wt.) collected from three aquatic sites at Fayoum Governorate, Egypt.

Studied sites	Exoskeleton	Gills	Muscles P.l.=0.5 ppm
Tanala	1.86± 0.2 ***	2.39±0.1***	2.28±0.1***
Elaam	0.72±0.1***	3.52±0.1***	4.48±0.1***
Elkaaby	10.8±0.1***	10.9±0.1***	7.68±0.1***
F- value	5153***	3919***	9272***

Permissible limits according to **WHO (1993).** Data are represented as an average of five samples ± SE, *** Highly significant (P<0.001).

Table (6): Meat quality of *Procambarus clarkii* muscles collected from three sites at Fayoum governorate, Egypt

Studied sites	Moisture content%	Ash content%	Total protein%	Total lipids %	Calcium concn. mg/kg	Phosphorus concn. mg/kg
Tanala	65±0.1**	10.23±0.1***	17±0.01***	4.52±0.01***	70.5±0.1***	600±0.02***
Elaam	70±0.1**	12.43±0.1***	13±0.01***	3.22±0.01***	66.5±0.1***	540±0.02***
Elkaaby	75±0.1**	15.33±0.1***	10±0.01***	2.52±0.01***	46.25±0.1***	530±0.04***
F- Value	52.000***	7260***	3700***	330900***	66367***	227279***

Data are represented as means of five samples ± S.E.

Highly significant different at p<0.001

Fig. (1): Accumulation factor of copper in muscles of *Procambarus clarkii* collected from three sites at Fayoum Governorate, Egypt.

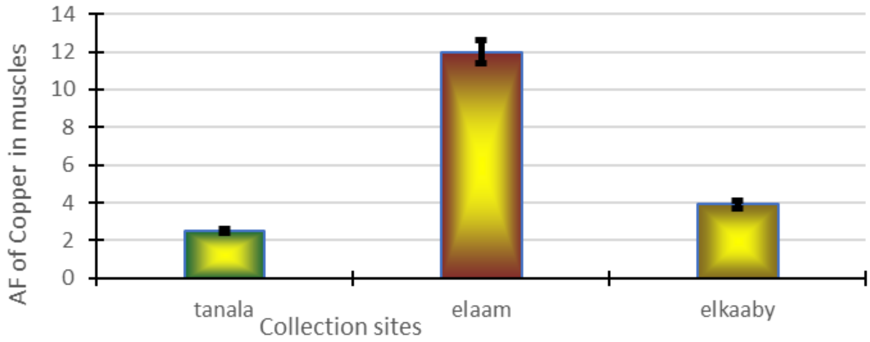


Fig. (2): Accumulation factor of zinc in muscles of *Procambarus clarkii* collected from three sites at Fayoum Governorate, Egypt.

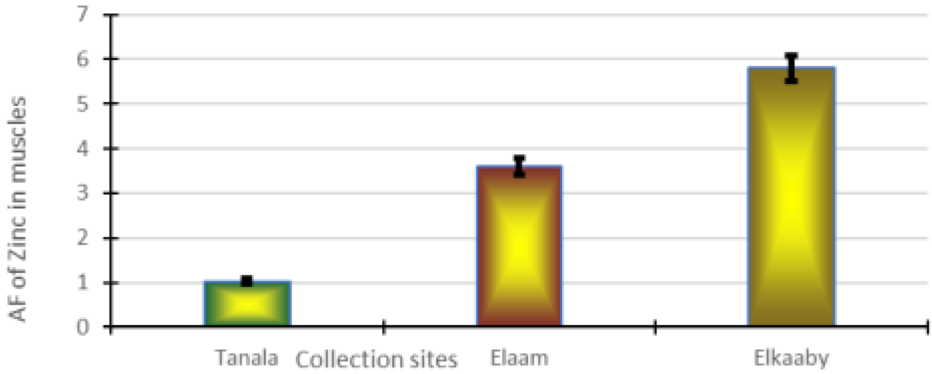


Fig. (3): Accumulation factor of lead in muscles of Procambarus clarkii collected from three sites at Fayoum Governorate, Egypt.

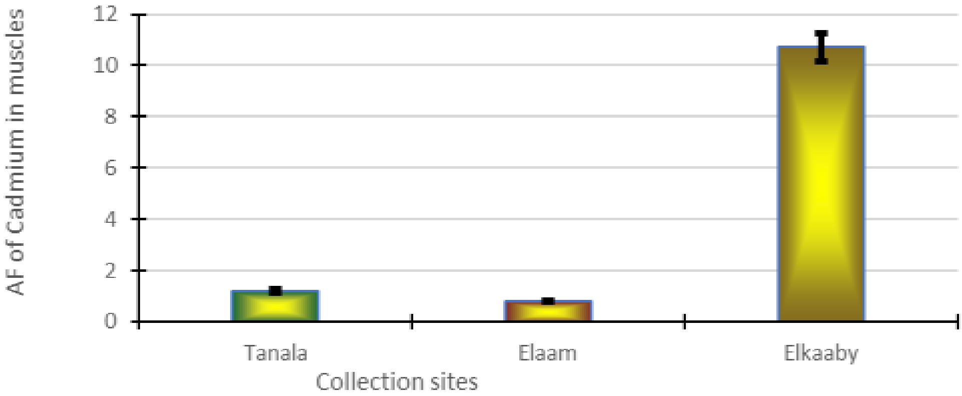


Fig. (4): Accumulation factor of cadmium in muscles of Procambarus clarkii collected from three sites at Fayoum Governorate, Egypt.

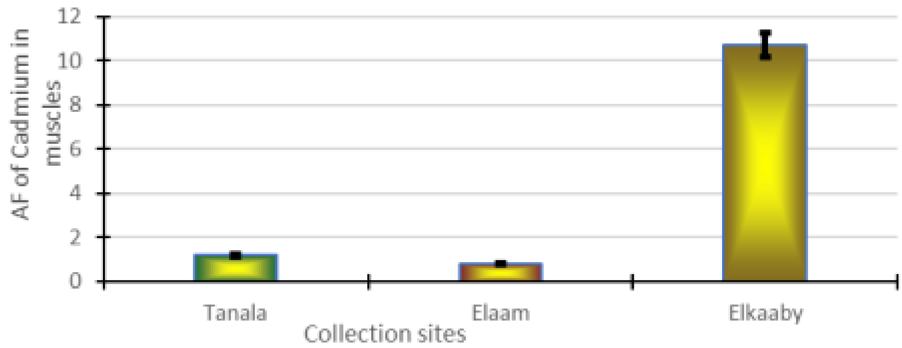


Fig. (5): Copper human risk consumption of edible crayfish muscles of Procambarus clarkii collected from three aquatic sites at Fayoum Governorate, Egypt.

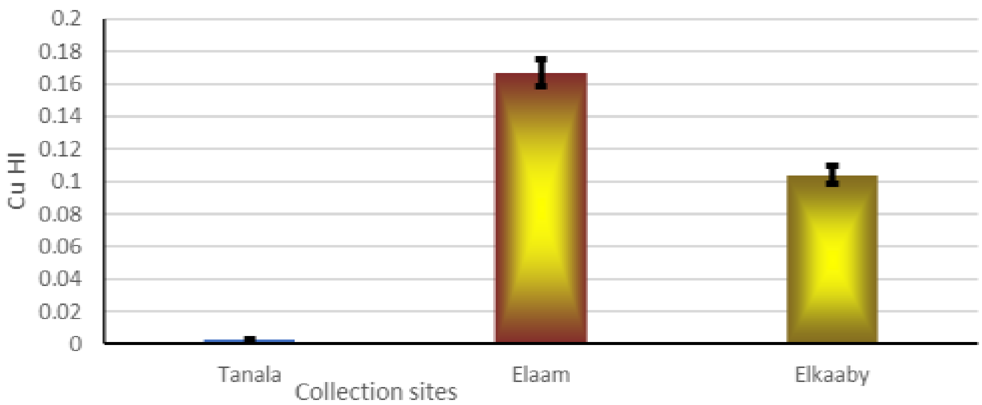
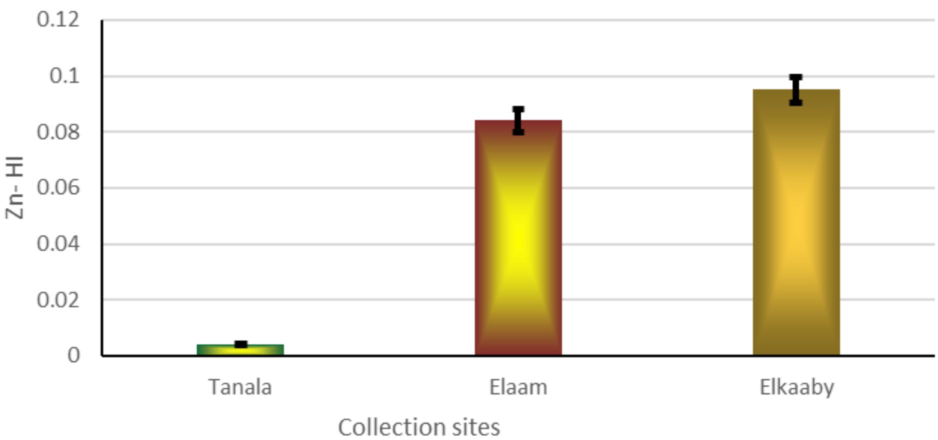


Fig. (6): Zinc human risk consumption of edible crayfish muscles of Procambarus clarkii collected from three aquatic sites at Fayoum Governorate, Egypt.



Z

Fig. (7): Lead human risk consumption of edible crayfish muscles of Procambarus clarkii collected from three aquatic sites at Fayoum Governorate, Egypt.

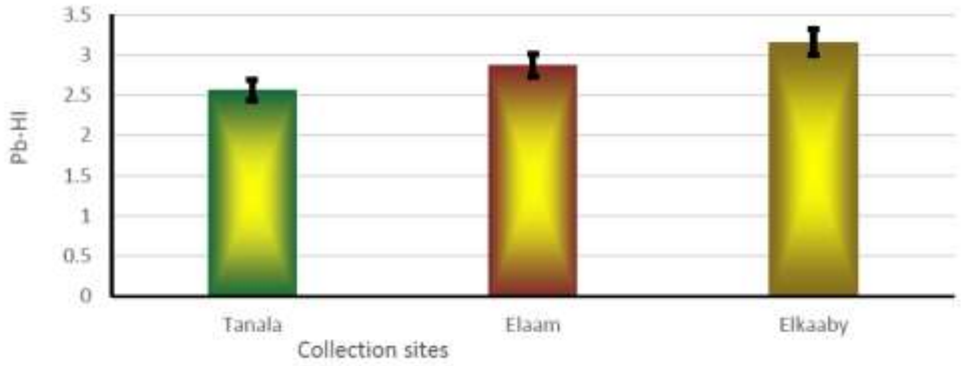
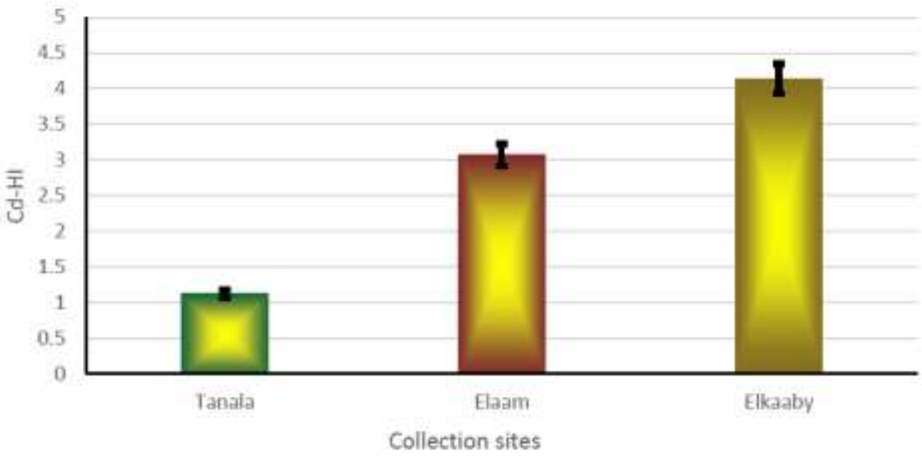


Fig. (8): Cadmium human risk consumption of edible crayfish muscles of Procambarus clarkii collected from three aquatic sites at Fayoum Governorate, Egypt.



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