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The use of freshwater crayfish *Procambarus clarkii* as an indicator of the bioavailability of some heavy metals in different watercourses in Egypt and the risk assessment of these metals

Soad S. Abdel Gawad[1], Awaad A. M. El-Saied[2], Neveen H. Mahmoud[3], Faten A. El-Fiqy[3], Eman A. Shaaban[1]

1-National Institute of Oceanography and Fisheries, Cairo, Egypt.
2- Zoology Department, Faculty of Science (Boys), Al-Azhar University, Cairo.
3- Zoology Department, Faculty of Science (Girls), Al-Azhar University, Cairo.

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ABSTRACT

The present work is the second to the first one titled "Bioaccumulation of Cadmium and Lead in the freshwater crayfish *Procambarus clarkii* from the River Nile, Egypt"(Shaaban *et al*., 2017). Crayfish *Procambarus clarkii* has been selected to assess some heavy metals accumulation pattern. This part aims to evaluate Iron, Zinc and Copper bio-accumulation in exoskeleton, muscles, hepatopancreas and ovary of this species as well as in water and sediment from the same sites for four seasons. Also, determination of risk assessment of Cadmium, Lead, Iron, Zinc and Copper. The results showed, Fe levels were higher than Zn and Cu concentrations in water, sediment and crayfish's organs in all sites. In water, the highest value of Fe was 1628.51µg/l recorded in El-Rayah El-Monoufy during summer, while Zn recorded highest value (60.6 µg/l) in spring at El-Rahawy. The highest value of Cu was 28.2µg/l at El-Warraq during summer. In sediment, Fe recorded the highest value (7005.5 µg/g) at El-Warraq during summer. Zn and Cu recorded the highest values (178.5 and 49.1 µg /g) at El-Rahawy during autumn and summer, respectively. In crayfish tissues, Fe was highest in the exoskeleton 126.13 µg/g at Helwan in summer while Cu and Zn were 37.17 and 43.74 µg/g respectively at El-Rahawy in autumn. On the other hand, the lowest concentrations of Fe, Zn, Cu were recorded in crayfish muscles (82.35, 37.85 and 17.14 µg /g respectively). For edible muscles, the results showed that, the values of Hazard index were within safe limits for Zn, Fe and Cu at all sites, while Cd values were detected only in El Rayah El Monoufy in safe limit. For Pb, HI values were unsafe at all sites. These results indicated, a larger amounts of crayfish will need to be eaten for health risk to be associated with Fe, Zn, Cu.

INTRODUCTION

In Egypt as it is well known that, water of the Nile is subjected to several sources of pollution in spite of all pollution control programs. Industrial and domestic wastewater and return drainage of irrigated water represents the important sources of pollution (Goher *et al*., 2014). Freshwater crawfish *P. clarkii* accumulates heavy metals from water and sediments in which it lives. Heavy metals bioaccumulation in aquatic animals is an important phenomenon in ecotoxicology and many researchers documented this fact (Abdel Gawad, 2005; Martins *et al*., 2011).

The pollution of sediments, water resources, and biota by heavy metals is of important especially in industrialized countries because of their toxicity, persistence and bio concentration nature (Ikem *et al*., 2003). In irrigation and drainage canals the major source of heavy metals is the discharge of domestic waste water containing high concentration of metals as: Al, Cu, Fe, Pb and Zn (APHA, 1995).

Heavy metal concentrations exist in low levels in water and attain considerable concentration in sediments and biota (Rashed, 2001). Iron, Zinc and Copper ions are essential elements for living organisms and aquatic plants when they are present in trace concentrations but they become toxic in high concentration (Kotickhoff, 1983; Abdel Gawad, 2018a; Abdel Gawad, 2018b).

This study aims to evaluate Iron, Zinc and Copper bio-accumulation in exoskeleton, muscles, hepatopancreas and ovary of Freshwater crayfish *P. clarkii* as well as in water and sediment in which it lives for four seasons from different water courses in Egypt. Also, it aims to determine the Hazard Index (HI) of Fe, Zn, Cu, Cd and Pb to obtain the health risk for the ingestion of this fish by human.

MATERIALS AND METHODS

Samples collection

Crayfish, *P. clarkii* were collected seasonally in the period from summer 2015 to spring 2016 from four main sites comprised: the main River Nile at (Helwan) and El-Warraq (El-Giza), in addition to El-Rahawy and El-Rayah El-Monoufy at El-Qalyubia and El-Monoufyia Governorates, respectively. Sample of ten crayfish individuals were taken randomly from each site every season. Each sample was washed with tap water to remove any adhering contaminants and then transported in an icebox to the laboratory. Crayfish samples were re-washed again with potable water (indiveduals ranged from 6.6 to 14.3cm length and 26.8-34.5gm weight), then skinned and dissected to obtain exoskeleton, muscles, hepatopancreas and ovary for heavy metals analysis.

Water samples were taken from the same four sites at the same time, from the subsurface layer and preserved with few drops of concentrated nitric acid and put in refrigerator for analyses.

Sediment were collected from bottom of the same sites during the same period using Ekman-Grab Sampler (15 x 15cm) and were kept in polyethylene bags and then transferred to laboratory for analyses.

Analytical methods:

Heavy metals determination in crayfish organs:

0.5 g of dried crayfish tissue samples of exoskeleton, muscles, hepatopancreas and ovaries (each alone) were digested according to Kotze *et al*., 2006). The samples were stored in clean plastic bottles prior to the determination of Fe, Zn and Cu concentrations using an atomic absorption reader (Savant AAS with GF 5000 Graphite Furnace).

Heavy metals determination in water samples:

Water samples of 500 ml were digested and filtered. Fe, Zn and Cu metals were analyzed by the same apparatus.

Heavy metals determination in sediment samples

Sediment samples were collected and oven dried at 60 °C to constant weight, homogenized in a mortar. Digestion and measure of heavy metals in the samples were conducted according to Shaaban *et al*. (2017).

Bio concentration factor

The bio concentration factor (BCF) was calculated according to Gobas *et al*. (2009).

Risk assessment for human consumption

According to the United States Environmental Protection Agency (USEPA, 2000) Risk assessment for human consumption of edible tissues was determined using the following equation ADD (mg/kg/day) = (C_m x IR / BW)

ADD= average daily dose

Cm= is the mean concentrations in edible organs (mg/kg dry weight),

IR= is the ingestion rate (0.0312 and 0.1424 kg/day for normal and subsistence animal consumers (fish, mollusks…), respectively, BW= is the body weight (assumed as 70 kg for normal adults), and: Then, risk was assessed by calculated the hazard index (HI) which means index of adverse effects from intake of specific contamination in food.

HI = ADD/ oral RfD USEOA (2000)

Oral RfD is the oral reference dose of the metal (mg/kg/day) based on the safe upper level of metals oral intake for an adult human. For example, the oral RfD for Cu, Zn, Fe and Cd is 0.04, 0.3, 0.7 and 0.001 mg/km/ day, respectively (USEPA, 2015) and 0.003 for Pb (WHO, 2011).

HI values <1.0 indicates that adverse health effects are not likely to occur. Hi ≥ 1, it may be presumed that adverse health effects are expected to occur.

Statistical analyses:

SPSS programs were used for Statistical analyses.

RESULTS AND DISCUSSION

Iron concentrations

Iron levels in water

Iron is an essential trace element found in drinking water in significant concentration. Usually, Iron finds in ground water in the form of ferric hydroxide (Oyeku and Eludoyin, 2010). The status of Iron in solution depends on environmental conditions, especially oxidation and reduction (Chapman, 1996).

Table (1) shows seasonal variations and annual average values of Iron concentrations in water samples collected from the studied sites. The average annual value was 412.01±239.20 µg/l. It was varied between sites, the maximum average of 648.4±659.26 µg/l was recorded at El-Rayah El Monoufy, decreased gradually to 578.7±703.68 at El-Rahawy and 268.2±183.75µg/l at El-Warraq and reached its minimum 152.63±33.42 µg/l at Helwan.

The Seasonal fluctuations of Iron varied between 126.37-1628.51, 147.94-392.22, 165 - 437.4, and 96.2 – 1620 µg/l during summer, autumn, winter and spring, respectively. The lowest values of Iron recorded during autumn may be due to its adsorption on the large amounts of organic matter and also to high concentrations of dissolved oxygen leading to oxidation of Iron from +2 to +3 (Masoud *et al.,* 2005). Fe concentration increased to the maximum value reached 1628.51 µg/l in summer at El-Rayah El-Monoufy. The elevated Iron concentration may be attributed to the release of Iron from sediment during the dissolution of Iron and may be due to agricultural and domestic effluents which loaded with high concentrations of Iron and may be due to the breakdown of organic matter and dead microorganism that releases the metal into the water (Price, 1976 and Elewa *et al*., 1996).

Iron levels in sediment

Table (1) shows the seasonal variations of Iron concentrations in sediment from different investigated areas. It has an annual average of 6089.366875±365.94 µg/g for all sites. The annual averages declined gradually from the maximum value of 6470.2625±391.05 µg/g at El-Warraq to the minimum of 5677.52±875.27µg/g at Helwan. Seasonal fluctuations in Iron concentrations values for all investigated area ranged between 5242.9-7005.51, 6518.02-6685.04, 5618-6184.32and 4716.72-6173.2 µg/g during summer, autumn, winter and spring, respectively. The obtained results indicate that the concentration of metals in the River Nile sediment show difference of concentrations according to their abundance in water and to seasons effect. Hamed (1998) stated that sediment acts as a reservoir for all the pollutants and dead organic matter coming from the ecosystem above. Hickling (1962) reported that the broken-down organic matter exists as "humus" which behaves like an organic compound of high molecular weight. Sediment with large amounts of humus has a very high capacity of adsorption. It can strongly adsorb different anions, cations and phosphate.

Table 1: Seasonal fluctuations of Iron concentrations in water (µg/l) and in sediment (µg/g) collected from the studied sites. ---- = not collected

Sites	Seasons	Summer	Autumn	Winter	Spring	Annual mean± SD
Water (µg/l)	Helwan	188.16	147.94	-----	121.8	152.63±33.42
	El-Rahawy	126.37	392.22	176.4	1620	578.7±703.68
	El-Warraq	513.47	298.26	165	96.2	268.2±183.75
	El-Rayah el Monoufy	1628.51	224.28	437.4	303.6	648.4±659.26
	Averages ± SD	614.12 ± 697.24	265.67 ± 104.32	259.6 ± 154.08	535.4 ± 728.93	412.01±239.20
Sediments (µg/g)	Helwan	5242.9	6685.04	-----	5104.62	5677.52±875.27
	El-Rahawy	6755.41	6596.86	5618	4716.72	5921.7475±947.42
	El-Warraq	7005.51	6518.02	6184.32	6173.2	6470.2625±391.05
	El-Rayah el Monoufy	6642	6647.73	5843.4	6018.62	6287.9375±418.31
	Averages ± SD	6411.45 ± 793.69	6611.9 ± 72.27	5881.9 ± 285.11	5503.29 ± 705.21	6089.366875±365.94

Iron levels in crayfish's organs

Crayfish accumulate toxic chemicals such as heavy metals which may reach hundreds or thousands of times above those in the water, sediment and food (Osman ***et** al.*, 2007). The ability of each tissue to regulate or accumulate metals can be directly attributed to the amount of metal concentrated in that tissue. Furthermore, physiological differences and tissue position in the fish can also affect the bioaccumulation of a definite metal (Kotze, 1997).

Levels of Iron varied from 76.12 - 126.13 µg/g, averaged 97.11±8.48 µg/g in the exoskeleton, declined gradually to 32.86 -104.10µg/g with an average of 72.54±9.29 µg/g in the hepatopancreas, 30.17-104.43 µg/g, with average of 70.66±12.24 µg/g in ovary and 34.18 - 82.35 µg/g with an average of 54.62±7.14 µg/g in muscles.

The highest concentrations of Iron in crayfish's organs were 126.13, 104.43 and 82.35 µg/g in exoskeleton, ovary and muscles respectively recorded at El-Rahawy during autumn and 104.10 µg/g in hepatopancrease at Helwan during winter (Table 2).

The annual averages of Fe concentrations indicated that, exoskeleton > hepatopancreas > ovary > muscles. These results agree with studies on the freshwater and marine decapods and other crustaceans (Baden *et al*., 1999). Anderson *et al*. (1997) reported abdominal muscle of the crayfish not accumulate metals after putting it in water receiving petroleum-laden effluents for 7-days. Abdel-Baky and Zyadah (1998) reported that muscles of marine fishes collected from Lake Manzala had low tendency to concentrate heavy metals than liver and gills. El-Deek *et al*. (1994) and Khallaf *et al*. (1998) stated that muscle tissue of fish is usually low in trace metal content.

It was noted that, Iron was accumulated in high concentrations in all organs and considered the most abundant of all measured metals. These results in agreement with the results of (Ghannam *et al*., 2014) in El-Bahr El-Pharaony Drain as well as the results of (Gilbert and Avenant-Oldewage, 2014) in the Vaal Dam, South Africa.

Table 2: Seasonal variations of Iron concentrations (μg/g) in different organs of *Procambarus clarkii* at different sites. ---- = not collected

Sites	Seasons	Summer	Autumn	Winter	Spring	Annual average ±SD
Exoskeleton	Helwan	120.24	103.08	-----	79.62	100.98±53.16
	El-Warraq	92.87	97.27	81.64	76.12	86.97±9.78
	El-Rahawy	99.92	126.13	93.17	106.85	106.52±14.21
	El-Rayah El-Monoufy	97.23	111.50	86.37	80.77	93.97±13.53
	Averages ±SD	102.56 ±12.13	109.49 ±12.53	87.06 ±43.78	85.84 ±14.14	97.11±8.48
Muscles	Helwan	55.26	59.33	-----	38.16	50.92±27.05
	El-Warraq	61.12	63.81	41.29	34.18	50.10±14.60
	El-Rahawy	61.34	82.35	53.17	64.18	65.26±12.31
	El-Rayah El-Monoufy	61.82	66.11	39.27	41.62	52.20±13.72
	Averages ±SD	59.88 ±3.09	67.90 ±10.03	44.58 ±23.11	44.54 ±13.44	54.62±7.14
Hepatopancreas	Helwan	104.10	57.51	------	32.86	64.82±43.85
	El-Warraq	70.50	73.47	67.61	48.19	64.94±11.42
	El-Rahawy	73.18	88.18	89.38	56.17	76.73±15.56
	El-Rayah El-Monoufy	100.41	79.92	81.19	73.22	83.69±11.68
Averages ±SD		87.05 ±17.65	74.77 ±12.98	79.39 ±40.69	52.61 ±16.80	72.54±9.29
Ovary	Helwan	96.70	72.21	------	30.17	66.36±43.07
	El-Warraq	67.25	60.53	63.00	40.18	57.74±12.03
	El-Rahawy	80.10	104.43	86.77	76.24	86.88±12.47
	El-Rayah El-Monoufy	82.06	74.00	62.08	68.42	71.64±8.48
Averages ±SD		81.53 ±12.05	77.79 ±18.73	70.62 ±37.11	53.75 ±22.06	70.66±12.24

Zinc concentrations

Zinc levels in water

Fish take Zn up directly from water, especially by mucous and gills (El-Naggar *et al*., 2009). It is an essential trace element found in river environment in the form of salts or organic complexes (WHO, 2003). Carbonell *et al.*, (1998), estimated the levels of these metals: Pb, Cd, Fe, Zn and Cu in water and found that they may not be lethal to the organisms but the concentration of such metals in their tissues creates hazards when used as food for human consumption.

Table (3) shows seasonal variations and annual average values of Zinc (Zn) concentrations in water collected from the studied sites. The average annual value was 19.9±6.95 μg/l for all investigated area. It was varied between sites, recorded the maximum average of 28.2±21.64µg/l at El-Rahawy, decreased to the minimum averages of 13.3±4.12 µg/l at El-Rayah–El-Monoufy, increased slightly to 15.06±4.99 µg/l at Helwan and 23.05 ± 22.65 µg/l at El-Warraq.

The Seasonal fluctuations in Zn values were varied between 8.8-56.6, 9.6- 17.6, 7.6 - 19.3, and 15.6– 60.6 µg/l during summer, autumn, winter and spring, respectively. Zn reached the maximum values in spring and summer. These results may be due to its adsorption on precipitated Fe $(OH)_3$ **(**Badr *et al*., 2006) and also may be due to reduced volume associated with higher evaporation rate induced by the higher water temperature during dry hot seasons (Obasohan and Eguavoen, 2008); and to the liberation of heavy metals from the sediment to the overlying water under decomposition of organic matter due to the high temperature and the fermentation process (Goher *et al*., 2014).

Zinc levels in sediment

Zinc may occur in sediment as the Zinc carbonate, Zzinc oxide and Zinc sulphide (Anon, 1978). Zn is involved in a variety of enzyme systems which contribute to energy metabolism, transcription and translation. Zinc is also potentially hazardous and excessive concentrations in soil lead to phytotoxicity as it is a weed killer (Abbasi *et al*., 1998).

Table (3) shows the annual and seasonal variations in Zinc concentrations in sediment from different investigated sites. Zn has an annual average of 133.4±5.4 µg/g for all sites, beings remarkably higher than those recorded in water. The annual averages declined gradually from the maximum value of 139.76±20.87µg/g at Helwan to the minimum one 127.35±37.58 µg/g at El-Rahawy.

Zinc values in the whole area ranged between 113.9-167.3, 126.9-178.5, 119.6-130.8 and 89.4-118.3 (µg/g) during summer, autumn, winter and spring, respectively. The highest value of Zinc concentrations was 178.5µg/g recorded at El-Rahawy during autumn, attributed to severe pollutants of domestic, agricultural and industrial pollution and also may be explained by Lumand Leslie (1983), who reported that the decrease of Zinc in water may be attributed to the removal of Zinc from the water column mediated by the decay of plankton populations and it's settling to the sediment declined sharply to only 89.4 µg/g during the following spring.

Table 3: Seasonal fluctuations of Zinc concentrations in water (µg/l) and sediment (µg/g) collected from different sites. --- = not collected.

Sites \ Seasons		Summer	Autumn	Winter	Spring	Annual mean ± SD
Water(µg/l)	**Helwan**	16.2	9.6	---	19.4	15.06±4.99
	El-Rahawy	15.4	17.6	19.3	60.6	28.2±21.64
	El-Warraq	56.6	11.6	7.6	16.4	23.05±22.65
	El-Rayah el Monoufy	8.8	11.2	17.9	15.6	13.3±4.12
	Averages ± SD	24.25±21.82	12.5±3.5	14.93±6.38	28±21.79	19.9±6.95
Sediments (µg/g)	**Helwan**	160	141	---	118.3	139.76±20.87
	El-Rahawy	113.9	178.5	127.6	89.4	127.35±37.58
	El-Warraq	167.3	126.9	130.8	117.6	135.65±21.81
	El-Rayah el Monoufy	144.7	135.5	119.6	112.3	130.83±66.82
	Averages ± SD	146.4±23.67	145.4±22.67	129.2±74.61	109.4±13.59	133.4±5.4

Zinc levels in crayfish's organs

The seasonal variations in Zinc concentrations in selected organs of crayfish, *Procambarus clarkii*, from the studied sites are presented in Table (4). These values were lower than those recorded in sediments. The results indicated also that Zinc concentrations in all organs except muscles were higher than concentrations recorded in water. These levels varied from 17.76-43.74 µg/g, averaged 26.56±3.32 µg/g in the exoskeleton, declined gradually into 12.83-31.50 µg/g with an average of 21.97±1.88 µg/g in the ovary, 9.86-32.44 µg/g with an average of 19.58±5.63 µg/g in hepatopancreas and 10.85- 37.85 µg/g with an average of 17.84±3.03 µg/g in muscles.

The obtained results from the annual averages of Zn concentrations indicated that, exoskeleton > ovary > hepatopancreas > muscles. The hepatopancreas accumulates Zn more than muscles because hepatopancrease is the principal organ of Zinc regulation. It can absorb excess Zinc from the stomach fluid and can remove excess Zinc if this is injected into the blood. Very little of the excess Zinc in the hepatopancreas can be lost in the urine or across the body surface. Zinc is lost only when the animal feeds and faeces are produced to which it can bind. As the amount of Zinc in the food increases, a smaller percentage of it is absorbed by the hepatopancreas and more is lost in the faeces (Utku GÜNER, 2010).

Table 4: Seasonal variations of Zinc concentrations (µg/g) in different organs of *Procambarus clarkii* at different sites.--- = not collected.

Sites	Seasons	Summer	Autumn	Winter	Spring	Annual average ±SD
Exoskeleton	Helwan	36.34	17.76	-----	35.16	29.75±17.13
	El-Warraq	18.75	34.18	21.16	25.96	25.01±6.80
	El-Rahawy	24.17	43.74	19.62	27.77	28.83±10.48
	El-Rayah El-Monoufy	27.96	19.73	22.86	19.98	22.63±3.82
	Averages ±SD	26.81±7.39	28.86±12.33	21.21±10.68	27.22±6.25	26.56±3.32
Muscles	Helwan	22.27	12.83	__	14.02	16.37±9.20
	El-Warraq	10.85	15.62	13.68	36.82	19.24±11.87
	El-Rahawy	37.85	17.38	16.38	13.62	21.31±11.14
	El-Rayah El-Monoufy	19.41	14.80	11.86	11.73	14.45±3.59
	Averages ±SD	22.59±11.26	15.16±1.88	13.97±7.22	19.05±11.88	17.84±3.03
Hepatopancreas	Helwan	30.42	18.75	__	22.17	23.78±12.86
	El-Warraq	14.80	20.89	19.26	21.74	19.17±3.09
	El-Rahawy	21.88	32.44	22.86	17.26	23.61±6.37
	El-Rayah El-Monoufy	12.66	10.85	13.68	9.86	11.76±1.72
	Averages ±SD	19.94±8.02	20.73±8.91	18.60±10.03	17.76±5.71	19.58±5.63
Ovary	Helwan	23.81	16.77	__	30.55	23.71±13.12
	El-Warraq	12.83	19.90	31.50	22.20	21.61±7.70
	El-Rahawy	17.89	29.77	19.26	25.40	23.08±5.52
	El-Rayah El-Monoufy	14.27	12.83	22.92	27.84	19.46±7.14
	Averages ±SD	17.20±4.89	19.82±7.23	24.56±13.30	26.50±3.55	21.97±1.88

Copper concentrations

Copper levels in water

Cu pollution throughout the world is increasing due to mining and industrial activities and environmental contamination by Cu occurs from weathering of minerals, rocks and anthropogenic sources.

Table (5) shows seasonal variations and annual average values of Copper (Cu) concentrations in water samples collected from the studied sites. The average annual value was 5.5±4.48 µg/l for all sites. The Seasonal fluctuations varied between 1.09-28.2, 1.2- 2.8, 2.06 – 9.17, and 1.61 – 5.17 µg/l during summer, autumn, winter and spring, respectively. The highest value (28.2µg/l) recorded at El-Warraq during summer may be due to domestic sewage and drain water where the domestic sources are the major contributors of Copper in the environment (Masoud *et al*., 2005). In general, the results showed slightly increase of Copper concentrations during the hot seasons than cold season and this may be attributed to the high evaporation rate of water during hot seasons and to the release of Cu from sediment to overlying water (Abdo, 2002).

Copper levels in sediment

Table (5) shows the annual and seasonal variations in Copper concentrations in sediment from different investigated areas. The annual averages declined gradually from the maximum value of 30.05±14.34µg/g at El-Rahawy to the minimum one, beings 13.9±5.61µg/g at El-Rayah El Monoufy with an annual mean of 20.6±6.85µg/g for the whole area. Seasonal fluctuations in Copper concentrations ranged between 19.483-49.102, 10.959-16.472, 9.26-31.62 and 13.64-26.34 µg/g during summer, autumn, winter and spring, respectively. Generally, the increase in Copper content in the sediment than the Copper content in water may be attributed to the removal of Copper from the water column mediated by the decay of the plankton or due to adsorption on the suspended matter or the complexation with organic matter leaving the water body to the sediment as reported in Muse River flowing through France, Belgium and Holland (NatherKhan and Lim, 1991).

Table 5: Seasonal fluctuations of Copper concentrations in water (µg/l) and sediment (µg/g) collected from the studied sites. --- = not collected

Seasons / Sites		Summer	Autumn	Winter	Spring	Annual mean ± SD
Water(µg/l)	Helwan	17.4	1.2	-----	2.13	6.91±9.09
	El-Rahawy	2.4	1.4	4.2	2.16	2.54±1.18
	El-Warraq	28.2	2.8	9.17	5.17	11.3±11.45
	El-Rayah el Monoufy	1.09	1.41	2.06	1.61	1.5±0.40
	Averages ± SD	12.27±12.94	1.7±0.73	5.14±3.64	2.7±1.62	5.5±4.48
Sediments (µg/g)	Helwan	19.483	16.472	-----	26.34	20.7±5.05
	El-Rahawy	49.102	15.312	31.62	24.17	30.05±14.34
	El-Warraq	23.202	11.75	19.26	17.26	17.8±4.76
	El-Rayah el Monoufy	21.918	10.959	9.26	13.64	13.9±5.61
	Averages ± SD	28.4±13.86	13.6±2.68	20.04±11.20	20.3±5.91	20.6±6.85

Copper levels in crayfish's organs

Copper (Cu) is plentiful in the environment and essential for the normal growth and metabolism of all aquatic organisms (Zia and Alikhan, 1989).

The seasonal variations in Copper concentrations in selected organs of crayfish, *Procambarus clarkii,* from the studied sites are shown in Table (6). Results showed that, the annual levels of Cu concentrations averaged 19.00±3.86 µg/g in exosekelton, 14.14±2.91µg/g in hepatopancreas, 12.90±2.02 µg/g in ovary and 10.06±1.51µg/g in muscles. These values were higher than those recorded in water and sediments. These levels varied from 11.64-37.17 µg/g in the exoskeleton, 6.91-28.60 µg/g in the hepatopancreas, 6.19-22.92 µg/g in the ovary and 5.92- 17.14 µg/g in muscles in the whole area of study.

The highest concentrations of Copper in crayfish's organs were 37.17, 28.60, 17.14 µg/g in exoskeleton, hepatopancrease and muscles respectively at Helwan in summer and 22.92 µg/g in ovary at El-Rayah El-Monoufy during winter.

From the annual averages levels of Cu concentrations, it was indicated that, exoskeleton > hepatopancreas > ovary > muscles. The lowest concentration of Cu accumulation was measured in the crayfish muscles. Exoskeleton and hepatoancrease

are the organs of Copper deposition. Similar results which assessed the Cu accumulation in fish have also been reported by many authors, e.g. Tepe *et al*. (2008) and Has-Schön *et al*. (2008). Copper was predominantly accumulated in the gills and hepatopancreas (Zia and Alikhan, 1989). As a whole, the toxicity of Cu can be mitigated by the presence of humic matter in the aqueous environment a sit complexes Cu (Nor**,** 1987). It can combine with other contaminants such as ammonia, Mercury and Zinc to produce an additive toxic effect on fish (Yacoub, 2007).
From the above results it was observed that the abundance of heavy metals in crayfish organs followed the order: Fe > Zn > Cu. Similar results were shown by Yacoub and Gad (2012), who found that the abundance of heavy metals in fish organs followed the order: Zn > Cu. The present work demonstrates that the exoskeleton displays the highest accumulation potential for all elements analyzed, while muscles have the lowest accumulation capacity; these observations are in harmony with Ghannam *et al*., 2014b, Khallaf *et al*., 1998.

It was observed that the Cu concentration in muscles was higher than in water which agree with Abou-El-Ezz and Abdel-Razeq (1991)who found that the concentration of Cu in muscles are much higher ten times in some cases, than that found in the surrounding water.

Table 6: Seasonal variations of Copper concentrations (μg/g) in organs of *Procambarus clarkii* at different sites. --- = not collected

Sites	Seasons	Summer	Autumn	Winter	Spring	Annual average ±SD
Exoskeleton	Helwan	37.17	11.64	-----	13.64	20.82±15.57
	El-Warraq	12.83	18.32	14.08	15.89	15.28±2.38
	El-Rahawy	28.78	21.14	24.66	19.65	23.56±4.06
	El-Rayah El-Monoufy	14.80	13.81	17.69	19.15	16.36±2.48
	Averages ±SD	23.39±11.60	16.23±4.29	18.81±10.37	17.08±2.83	19.00±3.86
Muscles	Helwan	17.14	9.05	----	8.02	11.40±7
	El-Warraq	5.92	9.05	10.41	9.02	8.60±1.90
	El-Rahawy	11.60	11.50	12.07	10.18	11.34±0.81
	El-Rayah El-Monoufy	11.84	9.87	7.62	6.24	8.89±2.46
	Averages ±SD	11.62±4.58	9.87±1.15	10.03±5.34	8.36±1.66	10.06±1.51
Hepatopancreas	Helwan	28.60	10.36	-----	12.72	17.23±11.82
	El-Warraq	6.91	12.98	8.64	15.46	11.00±3.92
	El-Rahawy	19.04	15.04	16.32	13.28	15.92±2.42
	El-Rayah El-Monoufy	16.77	5.92	13.10	13.87	12.42±4.61
	Averages ±SD	17.83±8.90	11.08±3.93	12.6±7.08	13.83±1.18	14.14±2.91
Ovary	Helwan	11.02	13.49	_	6.19	10.23±5.94
	El-Warraq	8.88	13.95	21.33	10.22	13.60±5.58
	El-Rahawy	17.31	11.60	10.84	11.07	12.70±3.08
	El-Rayah El-Monoufy	15.79	8.88	22.92	12.67	15.06±5.94
	Averages ±SD	13.25±3.95	11.98±2.30	18.36±10.63	10.04±2.75	12.90±2.02

The bioconcentration factors (BCF)

From figure 1, the bio concentration factors (BCF) of Fe, Zn and Cu in the Crayfish were lowest in muscle than other organs at all sites. The highest values of bio concentration factors (BCF) of all detected metals were shown in exoskeleton.

The highest values of bio concentration factors (BCF) of Fe, Zn in all organs were calculated at Helwan and El Rahawy regions while for Cu the highest values in all organs were detected in El-Rayah El-Monoufy. For organs, there were highly significant differences in Fe bioaccumulations between different organs ($P<0.01$) except between ovary and hepatopancreas. For Zn Highly significant differences were detected between exoskeleton and both of muscles and hepatopancreas ($P< 0.01$), but significant ones were detected between exoskeleton and ovary and between muscles and ovary ($P >0.05$). High significant differences between annual values of Cu bioaccumulations between organs ($P> 0.01$) except muscles –ovary and muscle - hepatopancreas.

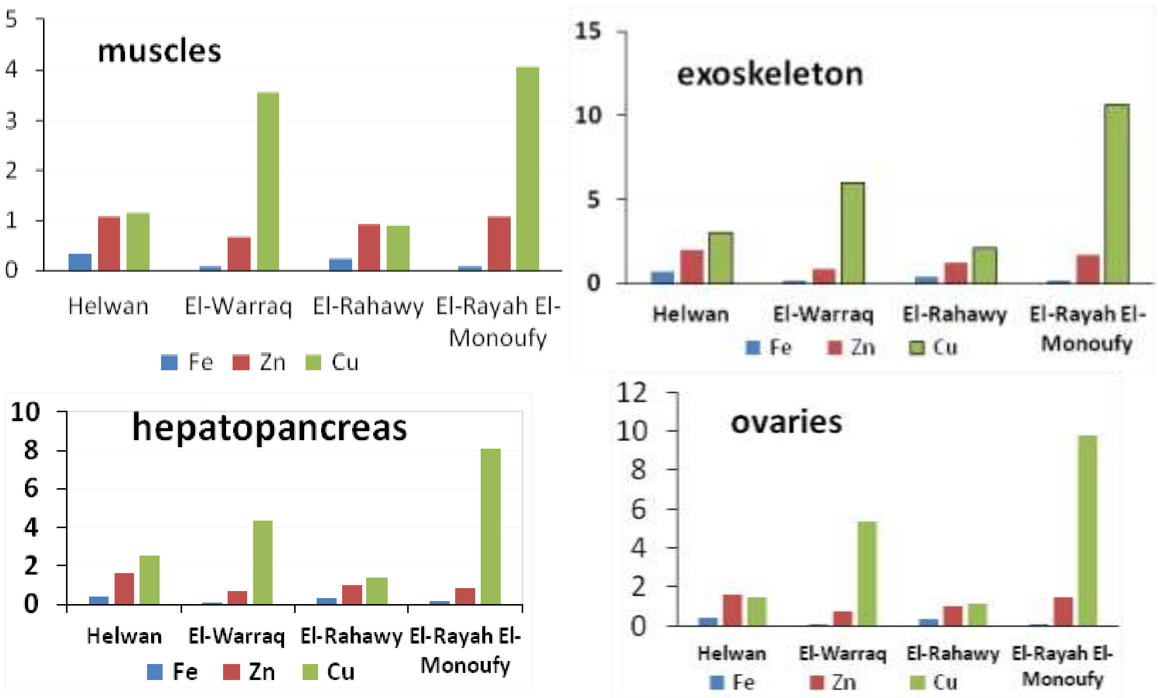

Fig. 1: Bio concentration factor of Iron, Zinc and Copper in muscles, exoskeleton, hepatopancreas and ovary of *P. clarkii* in different sites of investigation.

Risk assessment

The mean concentrations of estimated heavy metals in *P. clarkii* organs collected from the four studied sites were used to calculate the average daily dose (ADD) for normal and subsistence consumers. The calculated values of ADD are used to calculate the hazard index (HI) for each studied heavy metals. The calculated hazard index (HI) is an integrated risk calculation package that combines both the metal level in an animal edible tissues (fish, crustaceans, …etc) and the human consumption rate of these tissues to perform a risk characterization. In hazard identification, available data on biological endpoints are used to determine if a material is likely to pose a hazard to human health.

For exoskeleton, the values of annual HI were within safe limits ($HI <1$) for Fe and Zn at all sites. However, annual HI values for Cu were safe at all site except El-Rahawy. Cd had unsafe ($HI \geq 1$) at Helwan, El Warraq and El Rayah- El Mounofy, but undetected at El Rahawy. HI values for Pb were unsafe ($HI \geq 1$) and may be assumed had adverse health effects at all sites (Table 7).

For edible muscles, the values of HI were within safe limits for Zn, Fe Cu. Cd was detected only at El Rayah El Mounofy within safe limits at ingestion rate (HI

<1), but did not detect at other sites. These results indicate that a larger amounts of crayfish will need to be eaten for health risk to be associated with Fe, Zn. Cu. In contrast, Pb is the only metal that had high HI values ≥ 1 and considered had adverse effects on human health at all studied sites (Table 7).

These health troubles are very considerable such as renal failure, liver damage, cancer, damage to the nervous system, etc. all have been documented in human beings as a result of metal consumption (Malik *et al*., 2010).

In hepatopancreas, the HI values of Cu, Fe and Zn are within safe limits (HI< 1). The values of HI for Pb had high values greater than 1. Cd had HI value greater than 1 at Helwan, with adverse effect, but declined to <1 at El Warraq and El Rahyah El Mounofy and did not detected at El Rahawy.

In ovaries, the HI values of Cu, Fe and Zn were within safe limits (HI< 1). The values of HI for Pb had high values greater than 1 at all sites. Cd had HI value ≥ 1 at Helwan and El Rayah El Mounofy and with adverse effect, but decline to <1 at El Warraq and was not detectd at El Rahaway. This agree with Khalil *et al*. (2013) who reported that Pb and Cd exceeded the guidelines and the permissible limits in this species (Table 7).

Table 7: Annual average of Hazard Index (HI) of Fe, Zn, Cu, Cd, Pb for normal (N) and Subsistence (S) animal consumers.

Organs	Sites	Animal consumers	Annual average				
			Fe	Zn	Cu	Cd	Pb
Exoskeleton	I	N.	0.066	0.044205	0.231957	0.732	1.786667
		S.	0.293	0.201756	0.428556	3.343333	8.15
	II	N	.056	0.037162	0.170263	0.646057	1.803333
		S	0.25	0.169609	0.777097	2.95021	6.17725
	III	N	.069	0.042826	0.262498	nd	1.765
		S	0.31	0.195461	1.198067	nd	8.0525
	IV	N	0.06	0.033626	0.182325	0.585	2.0475
		S	0.271	0.15347	0.83215	2.665	9.34725
Muscles	I	N	0.032	0.024326	0.127066	nd	0.926667
		S	0.147	0.111027	0.579941	nd	4.23
	II	N	0.033	0.028589	0.095829	nd	1.1175
		S	0.145	0.130482	0.437371	nd	5.1115
	III	N	0.042	0.031657	0.126332	nd	1.175
		S	0.19	0.144485	0.576593	nd	5.37
	IV	N	0.033	0.021469	0.099088	0.135	1.305
		S	0.15	0.097985	0.452247	0.71	5.949
Hepatopancreas	I	N	0.044	0.03533	0.191954	0.14	1.096667
		S	0.188	0.161251	0.876099	0.65	5.006667
	II	N	0.04	0.028485	0.122544	0.7115	1.2575
		S	0.187	0.130008	0.559302	3.245	5.735
	III	N	0.049	0.035078	0.177394	nd	2.1275
		S	0.223	0.160098	0.809646	nd	9.7075
	IV	N	0.054	0.017476	0.138339	0.5306	1.475
		S	0.241	0.079761	0.631392	2.42	6.7275
Ovary	I	N	0.042	0.035226	0.114028	0.23	0.95
		S	0.193	0.160776	0.520438	1.04	4.33
	II	N	0.037	0.032103	0.151487	0.158333	1.0475
		S	0.169	0.14652	0.691403	0.719	4.7825
	III	N	0.055	0.03429	0.14157	nd	1.85775
		S	0.251	0.156504	0.64614	nd	8.4915
	IV	N	0.046	0.02892	0.167867	0.532333	1.4475
		S	0.207	0.131991	0.766163	2.41666	6.6175

Site I: Helwan, II: El-Warraq, III: El-Rahawy, IV: El-Rayah El-Menoufy, nd = not detected

According to USEPA (2012) no health risk may occur as a result of ingestion of the fish at total Hazard Index (HI) below one. Hence, 1 < HI < 10 means moderate hazard while greater than 10 means high hazard or risk (Ukoha *et al*., 2014).

Using the cumulative risk effects for normal and subsistence animal consumers, edible muscles values of HI had adverse effects on human health at all studied sites. This agrees with Khalil et al. (2013) consumption of these animals in a large quantities for a long time or from contaminated areas, could cause adverse health results.

It can be concluded that, bioaccumulation of heavy metals in *P. clarkii* in muscles was within the standards guidelines except in highly polluted area and it is recommended that, reconsideration in eating this fish.

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