International Journal of Waste Resources

El Assal and Abdel-Meguid, Int J Waste Resour 2017, 7:2
DOI: 10.4172/2252-5211.1000270

Research Article Open Access

Impact of Heavy Metal Pollution on *Procambarus clarkii* (Crustacea: Decapoda), from Egypt

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Abstract

The present study was conducted to assess the accumulation of some metals (Fe, Cd, Cu, Pb, Mn, Mg, Ca and Zn) in the River Nile water and sediment, at four sites (Gezyrat Al- Warrak (site I), Manial Sheeha (site II), Al-Hawamdia (site III) and Helwan (site IV), at Great Cairo, as well as in the exoskeleton, hepatopancreas , muscles and gills of the crayfish *Procambarus clarkii*, collected from the same sites). The results obtained show that the different concentrations of the metals in the Nile water were in the descending order Mg>Zn>Fe>Cu>Mn>Pb>Cd, at all studied sites. Fe and Zn concentrations were higher than the permissible limits, while the remaining metals were within the allowable levels. Whereas, the concentrations of metals in the sediment showed different patterns, according to their abundance in water. The abundance of these metals in the sediment was in the order Fe>Mg>Ca>Zn>Mn>Cu>Pb>Cd, at sites I and II, Fe>Mg>Ca>Zn>Mn>Cu>Cd>Pb, at site III and Mg>Fe>Ca>Zn>Mn>Cu>Cd>Pb, at site IV. Metal concentrations in the sediment were higher many folds than their values in the overlaying water. *P. clarkii* accumulated heavy metals in its tissues regardless their abundance in the water and/or sediment. The concentrations of the selected metals in the crayfish muscles were lower than the international permissible levels. Relative to the allowable limits for metals in foods, there was no sufficient accumulation of any of the detected metals in the muscles of *P. clarkii* to indicate that no significant health hazard would result from the consumption of the muscle parts of the animal. This study suggests, also, that *P. clarkii* may be used as bioindicator for heavy metals pollution in the freshwater systems, as well accumulation of these pollutants can cause depuration of the accumulated metals by decreasing their amounts in water.

Keywords: Heavy metals; Crayfish; *Procambarus clarkii*; Crustaceans; Pollution; Exoskeleton; Gills; Hepatopancreas; Muscles

Introduction

Procambarus clarkii stands as an important food in many parts of the world, being a rich source of protein. It was introduced in the Egyptian waters in the early 1980s, from the USA, for aquaculture [1]. It is consumed in few areas, as it is cheaper than other protein source. The uprising input of pollutants into the aquatic ecosystems showed that they were the major factors affecting the biology of any living organism [2,3]. The uncontrolled discharges of domestic sewage, industrial effluents, pesticide, heavy metals, agricultural fertilizers into the freshwaters cause many dramatic problems to most of the aquatic fauna [4]. Heavy metals constitute a major problem because they are toxic and tend to accumulate in the body organs [5-10]. Aquatic animals may absorb dissolved metals from surrounding waters and sediments which may accumulate in various tissues in significant amounts and are eliciting toxicological effect at critical targets [2,5-10]. Some metals such as zinc, copper, manganese and iron are essential for the well-being and growth of living organisms including man. However, they are likely to show toxic effects at higher levels than normally required [11]. Other elements such as lead and cadmium are not essential for metabolic activities and exhibit toxic properties, delayed embryonic development, malformation and reduce the growth of aquatic animals [12]. Therefore, a study to assess the accumulation of heavy metals in the Nile water , sediment and in the crayfish *P. clarkii* tissues was carried out, in order to through light on the safeness of *P. clarkii* consumption as a source of protein, on the public health.

Materials and Methods

Samples of River Nile water were collected, at the depth of 1 meters from the surface, with a special device, from four sites : Gezyrat El-Warrak (Site I); Manial Sheeha (Site II); Al- Hawamdia (Site III) and Helwan (Site IV), at the great Cairo, Egypt. Acid was added to prevent loss of metals. Sediment samples were collected from the same sites, and 50 mg of each sample were accurately weighed and placed into a dry and clean Teflon digestion beaker. 2 ml of HNO_3, 2 ml of HCL and 6 ml of HF were added to the Teflon beakers. Samples were digested on a hot plate at 120-150°C for 40 minutes. The digest was then filtered and the filtrate transferred to 15 ml plastic tube and deionized water was added to appropriate level. A blank was prepared in the same manner.

Samples of the crayfish *P. clarkii* were collected, from the same four sites, using a 0.7 cm diagonal net size. Collected crayfish were transported immediately alive to the laboratory. Specimens were kept in fiber glass containers, 43 × 23 × 15.5 cm in dimensions, filled with dechlorinated tap water, at the depth of 12–14 cm. *P. clarkii* were dissected and the exoskeleton, gills, hepatopancreas and muscles were removed, washed, thoroughly with deionized water and refrigerated at – 20°C, till analysed for metals. For metal analyses, weighed tissue samples were digested in 65% concentrated pure nitric acid and hydrogen peroxide at a ratio of 4:1 [13], at elevated temperature and pressure by using Microwave Sample Preparation Labstation, MLS-1200 MEGA. Then, samples were converted to soluble matter in deionized water to appropriate concentration level. Metals were measured using atomic absorption spectrophotometer (model A-Analyst 100 Perkin Elmer instrumentation), according to IAEA [13]. Metals measured in

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Received June 01, 2017; **Accepted** June 19, 2017; **Published** June 26, 2017

Citation: El Assal FM, Abdel-Meguid ZA (2017) Impact of Heavy Metal Pollution on Procambarus clarkii (Crustacea: Decapoda), from Egypt. Int J Waste Resour 7: 270. doi: 10.4172/2252-5211.1000270

water, sediment and tissues were Lead (Pb), Copper (Cu), Iron (Fe), Mangnese (Mn), Magnesium (Mg), Zinc (Zn), Calcium (Ca) and Cadmium (Cd). A software computer GraphPad Instat was used to test the significance of the difference between mean values of experimental and control groups. To analyze more than two sets of data, ANOVA (one way analysis of variance) for parametric data was used, following Tukey-Kramer M.C.T (Figure 1).

Results

The concentration of Fe in the Nile water was significantly high at sites II and IV (P<0.001 and P<0.01, respectively) , whereas it was slightly elevated, at site III (P<0.05), in comparison to site I, used as control, as it was, more or less, the less contaminated site (Table 1 and Figure 2a). The concentration of the same metal in the sediment was also significantly high at sites II and III (P< 0.01 and P< 0.05, respectively), while it was slightly higher at site IV, as compared to site I (Table 1 and Figure 2b). Table 1 and Figures 2c-2f illustrates the concentrations of Fe in the different tissues of *P. clarkii*. The highest concentration of Fe was recorded in the exoskeleton, gills and hepatopancreas (P<0.01), at site II, followed by site IV for Fe in gills, then sites III and IV, for Fe, in the hepatopancreas. While, there was insignificant difference in the accumulation of Fe in the muscles of the crayfish.

The concentration of Cu in water was insignificantly higher at sites II, III and IV, compared to site I (Table 2 and Figure 3a). Whereas, in the sediment, it was significantly lower at site IV (P<0.05), but at sites II and III, an insignificant low Cu concentration was recorded , in comparison to site I (Table 2 and Figure 3b). Table 2 and Figures 3c-3f show the concentrations of Cu in tissues. The exoskeleton of *P. clarkii* revealed significant low value of Cu concentration, at sites III and IV (P<0.05 and<0.01, respectively), while, at site II it was insignificantly lower than the control site. In the gills, the concentration of Cu was significantly lower at sites II and IV, compared to site I, while, at site III, it was insignificantly higher. No great difference was reported in Cu accumulation in the muscles of the crayfish.

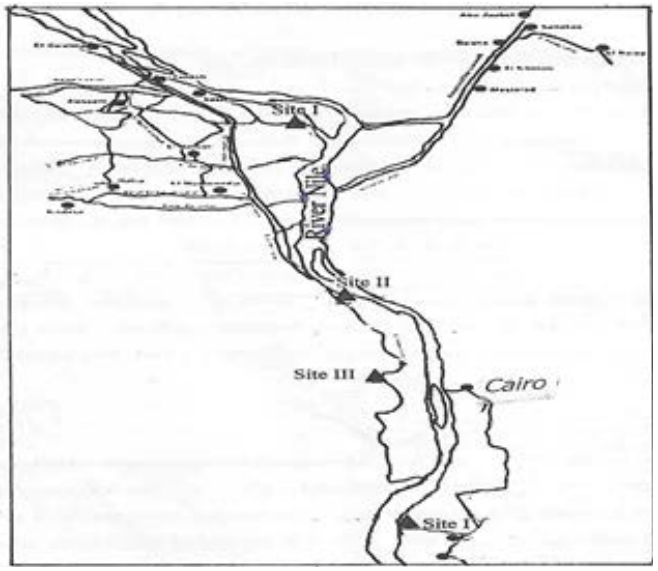

Figure 1: Representative map showing the different sample-sites along the River Nile, Egypt. The studied areas: Gezyrat El-Warak (Site-1), Manial Sheeha (Site-II), Al-Hawamdia (Site-III) and Helwan (Site-IV).

The concentration of Ca in water (Table 3 and Figure 4a) was significantly lower (P<0.001) at site II, whereas, it was significantly elevated, at sites III and IV (P<0.05 and P <0.001, respectively), in comparison to site I. In the sediment, Ca concentration was significantly lower (P<0.01), at sites II and III, than at the control site I and significantly higher at site IV (P<0.01) (Table 3 and Figure 4b). The concentrations of Ca in the different tissues of *P. clarkii* is shown in Table 3 and Figures 4c-4f. Ca concentration in the exoskeleton of *P.clarkii* showed significant increase, at sites II (P<0.01), compared to the control site, while, at site III a marked decrease (P<0.01) was observed and at site IV, an insignificant decrease was reported. Gills revealed variable pattern of Ca concentration. At sites II and IV, a significant decrease (P<0.05, respectively) was recorded, whereas at site III, Ca concentration increased insignificantly, as compared to site I. Also, in the hepatopancreas, Ca increased insignificantly at sites II an IV and decreased at site III, in comparison to site I. Ca in muscles was significantly higher at site II than at the control site, while at sites III and IV, it was more or less of the same value. The concentration of Zn in water was significantly higher at sites II, III and IV (P>0.01, respectively) than at site I (Table 4 and Figure 5a). In contrast, the sediment showed significantly lower values for the same sites (P<0.01, P<0.001 and P<0.001, respectively), as compared to site I (Table 4 and Figure 5b). Zn concentration in the exoskeleton of *P. clarkii* was significantly lower at site II and III (P>0.01, respectively) than at the control site, while, it was insignificantly lower, at site IV. As regard the concentration of Zn in the gills, site IV revealed significantly higher concentration (P>0.001) than at site I, while sites II and III showed lower values. significantly high difference of Zn was observed in the hepatopancreas at sites II and IV (P>0.001 and P<0.01, respectively), compared to site I. In muscles, Zn concentration was significantly lower at sites II and IV (P>0.01 and P>0.05, respectively) than at the control site (Table 4 and Figures 5c-5f).

Cd concentration in the water was markedly lower at sites II, III and IV (P< 0.001, respectively), in comparison to site I. Whereas, in the sediment of the same sites, significant increases in the concentration of Cd were observed (P<0.01, P<0.01 and P<0.001, respectively) (Table 5 and Figures 6a and 6b). In the exoskeleton of *P. clarkii*, significant increase of Cd was observed at sites II and IV (P<0.05 and 0.01, respectively), compared to the control site (Table 5 and Figure 6c). Also, in 0.001) was recorded at site IV but a significant decrease was observed at site III (Table 5 and Figure 6d). Cd concentration, in the hepatopancreas, decreased significantly at sites II and III (P<0.001, respectively) and site IV, it was more or less the same as at the control site (Table 5 and Figure 6e). No significant changes were recorded in the Cd concentration in the muscles of *P.clarkii*, at the four sites of the study (Table 5 and Figure 6f).

The concentration of Pb, in the Nile water, at sites II, III and IV was significantly higher (P<0.001, respectively) than at the control site (Table 6 and Figure 7a). Whereas, in the sediment, Pb decreased significantly (P<0.001), at sites III and IV and at site II, it was of a similar concentration as the control site (Table 6 and Figure 7b). The exoskeleton of *P. clarkii* showed significant low values of Pb, at sites II and III (P<0.001 and P< 0.01, respectively), compared to the control site, while, at site IV, a significant increase (P<0.001) was observed. In gills, the concentration of Pb was significantly lower (P<0.01) at site II than at the control site but was more or less similar, at sites III and IV. In the hepatopancreas and muscles, Pb concentration increased significantly, at site III (P<0.001). While, it decreased significantly (P<0.01), in the hepatopancreas, at site II , and was of the same value in the muscles, at sites II and IV, as compared to the control site (Table 6 and Figures 7c-7f).

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	Muscles
Gezyrat al-Warrak (Site I)	Mean	0.018	19395	188.3	289.8	154.58	48.88
	S.D.	0.003	1872	13.14	23.08	19.11	6.98
Manial Sheeha (Site II)	Mean	1.486***	28177**	376.6**	399.1**	341.4**	45.14
	S.D.	0.022	2132	67.23	61.42	35.81	8.67
Al-Hawamdia (Site III)	Mean	0.032	22145*	174.4	259.6	239.4**	45.8
	S.D.	0.007	1086	18.36	25.87	12.5	11.7
Helwan (Site IV)	Mean	0.147**	20567	145.9	408.8**	253.2**	42.58
	S.D.	0.015	950.4	25.01	19.2	17.00	13.69

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (*P<0.05, **P<0.01 and ***P<0.001)

Table 1: Concentration of iron (Fe) expressed as µg Fe/ml in water, µg Fe/g in wet weight sediment and in fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkii*.

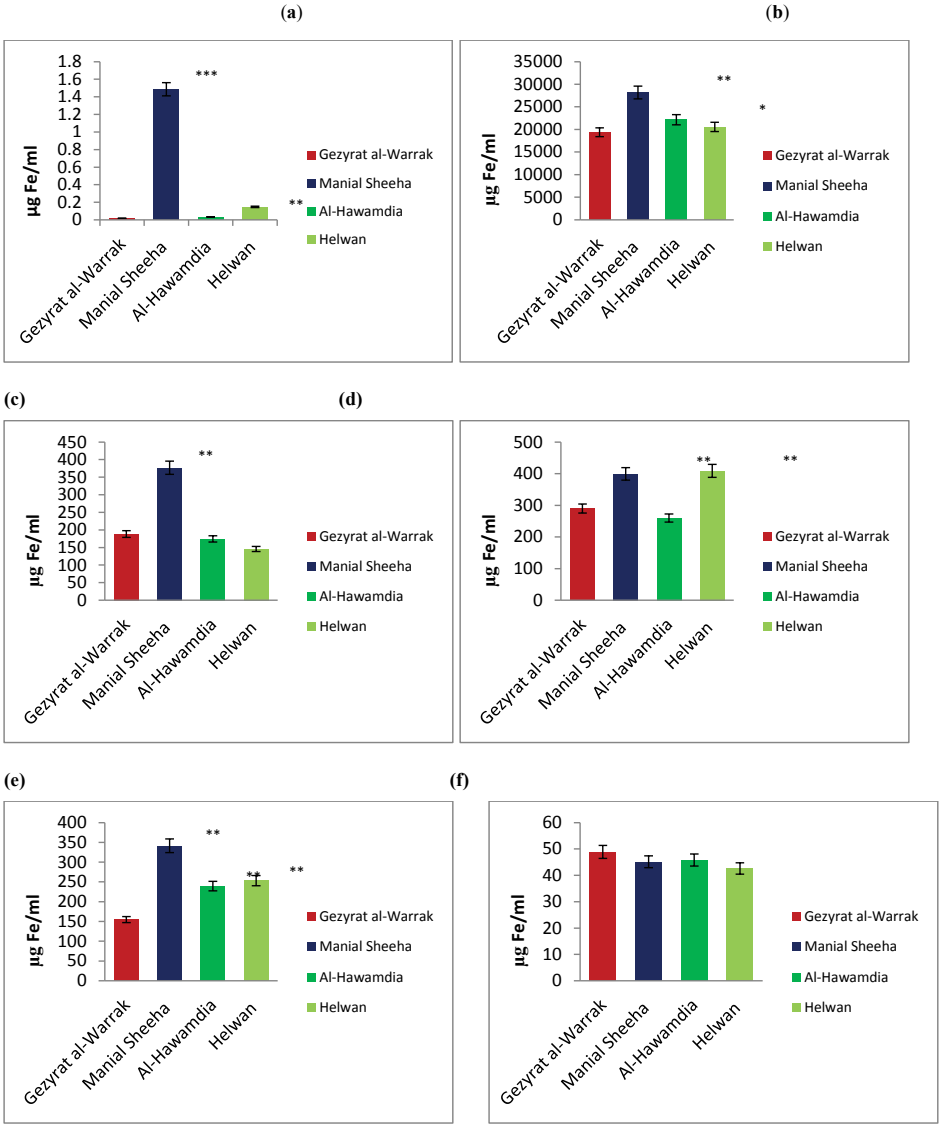

Figure 2: Iron concentration expressed as µg Fe/ml in water (a), µg Fe/g in wet weight sediment (b) and in fresh tissue of exoskeleton (c), gills (d), hepatopancreas (e) and muscles (f) of *P. Clarkii*, at the different sites of the investigation levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	Muscles
Gezyrat al-Warrak (Site I)	Mean	0.013	19.52	9.579	38.03	12.96	4.363
	S.D.	0.005	43.25	0.865	1.454	0.699	0.658
Manial Sheeha (Site II)	Mean	0.015	18.66	8.458	32.89*	48.83***	4.612
	S.D.	0.004	3.422	1.562	2.374	6.176	0.981
Al-Hawamdia (Site III)	Mean	0.015	16.21	7.852*	41.86	24.69***	5.502
	S.D.	0.003	2.312	0.958	3.876	3.923	0.505
Helwan (Site IV)	Mean	0.014	12.93*	6.119**	34.59*	35.58***	5.062
	S.D.	0.004	1.623	1.065	4.078	4.078	0.790

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (*P<0.05, **P<0.01 and *** P<0.001)

Table 2: Concentration of copper (Cu) expressed as µg Cu/ml in water, µg Cu/g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkii.*

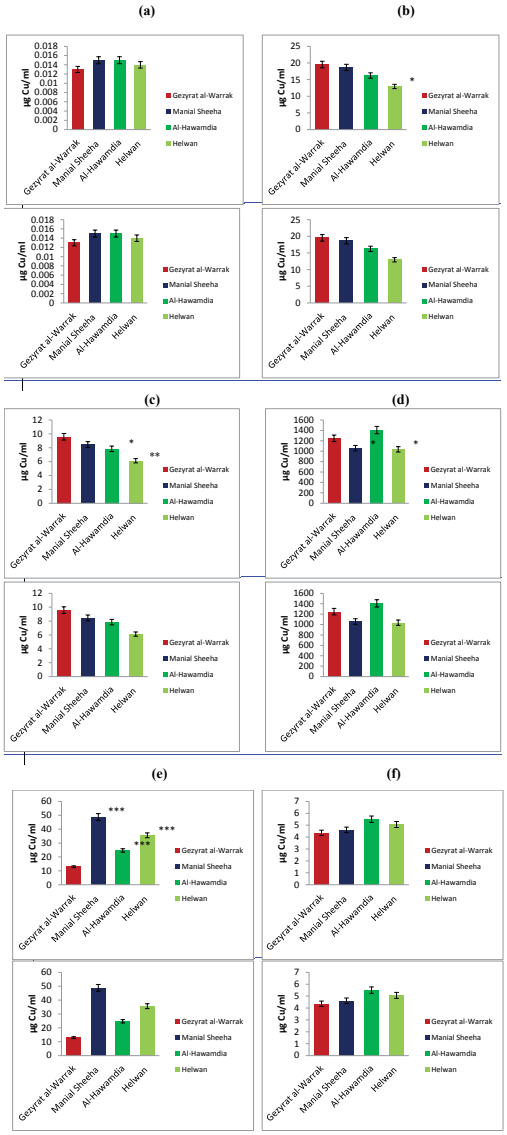

Figure 3: Copper concentration expressed as µg Cu/ml in water (a), µg Cu/g in wet weight sediment (b) and fresh tissues of exoskeleton (c), gills, hepatopancreas (e) and muscles (f) of *P. Clarkii* in the different sites of the investigation; levels of significance are *P<0.01, **P<0.01 and ***P<0.001.

Mn concentration in water was significantly higher, at sites II, III and IV (P<0.001, respectively), as compared to site I (Table 7 and Figure 8a). In the Nile sediment, a significant low value of Mn was observedat site II (P<0.001), while, at sites III and IV, significantly high concentrations were recorded (P<0.05 and P<0.001, respectively), as compared to the control site (Table 7 and Figure 8b). The concentration and the BCFs of Mn in the different tissues of *P. clarkii* is shown in Table 7 and Figures 8c-8f. Mn was considerably accumulated (P<0.001), in the exoskeleton of the crayfish, at site II, while a moderate value of the metal (P<0.05), was observed, in the same tissue, at site IV, compared to the control site. In gills, lower accumulation of Mn than in site I was recorded at site II (P<0.05), whereas, a slight increase in Mn concentration (P<0.05) was observed at site IV. The concentration of the metal decreased markedly (P<0.001), in the hepatopancreas of *P. clarkii*, at sites II, III and IV, respectively, as compared to the control site. Also, Mn accumulation, in muscles of the crayfish, decreased significantly (P<0.01), at sites II and IV.

The concentration of Mg in the River Nile water was significantly higher at sites III and IV than at the control site (P<0.001, respectively), while, at site II, it was insignificantly lower (Table 8 and Figure 9a). In the sediment, an increase in Mg concentration was observed, at sites II, III and IV, compared to site I (P<0.05, P<0.01 and P<0.001, respectively) (Table 8 and Figures 9b-9f) summarize the concentrations and BCFs of Mg in the different tissues of *P. clarkii*. The concentration of Mg in the exoskeleton increased significantly at sites II and III (P<0.05 and P<0.001, respectively). While, at site IV, it was similar to that of the control site. In gills, Mg concentration declined significantly at sites II, III and IV (P<0.001, P<0.001 and P<0.05, respectively). The concentration of Mg, in the hepatopancreas, showed similar value at site II as at site I, whereas, it was lower (P<0.05), at sites III and IV. In muscles, Mg concentration was significantly higher at sites III and IV (P<0.05 and P<0.001, respectively), but was insignificantly lower at site II, than at the control site.

Discussion

This study shows that the different concentrations of heavy metals under investigation, in the River Nile water, followed the same pattern: Ca > Mg > Zn > Fe > Cu > Mn > Pb > Cd, at the four sites of the study (Gezyrat al – Warrak (site I), Manial Sheeha (site II), Al- Hawamdia (site III) and Helwan (site IV)). The highest concentrations of Ca and Mg were recorded at site IV, whereas, that of Fe and Zn was observed at site II. Also, Cu showed the most elevated concentration at sites II and III, while the highest concentration of Cd was at site I, that of Pb at site III and that of Mn at sites II and IV. The concentrations of

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	muscles
Gezyrat al-Warrak (Site I)	Mean	37.94	8582	5.127	1248	394.7	459.6
	S.D.	5.423	426.7	0.101	117.2	43.5	101.5
Manial Sheeha (Site II)	Mean	18.53***	6832**	5.595**	1058*	406.5	663.4**
	S.D.	2.621	381.3	0.385	131.6	27.00	64.13
Al-Hawamdia (Site III)	Mean	46.11*	6233**	3.056**	1406	394.2	470.1
	S.D.	4.581	487.2	0.250	143.1	29.76	82.09
Helwan (Site IV)	Mean	64.73***	9757**	4.983	1036.4	444.6	485.7
	S.D.	4.691	612.3	0.331	133.15	58.07	117.67

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (*P< 0.05, **P<0.01 and ***P<0.001)

Table 3: Concentration of calcium (Ca) expressed as µg Ca/ml in water, µg Ca/g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkii*.

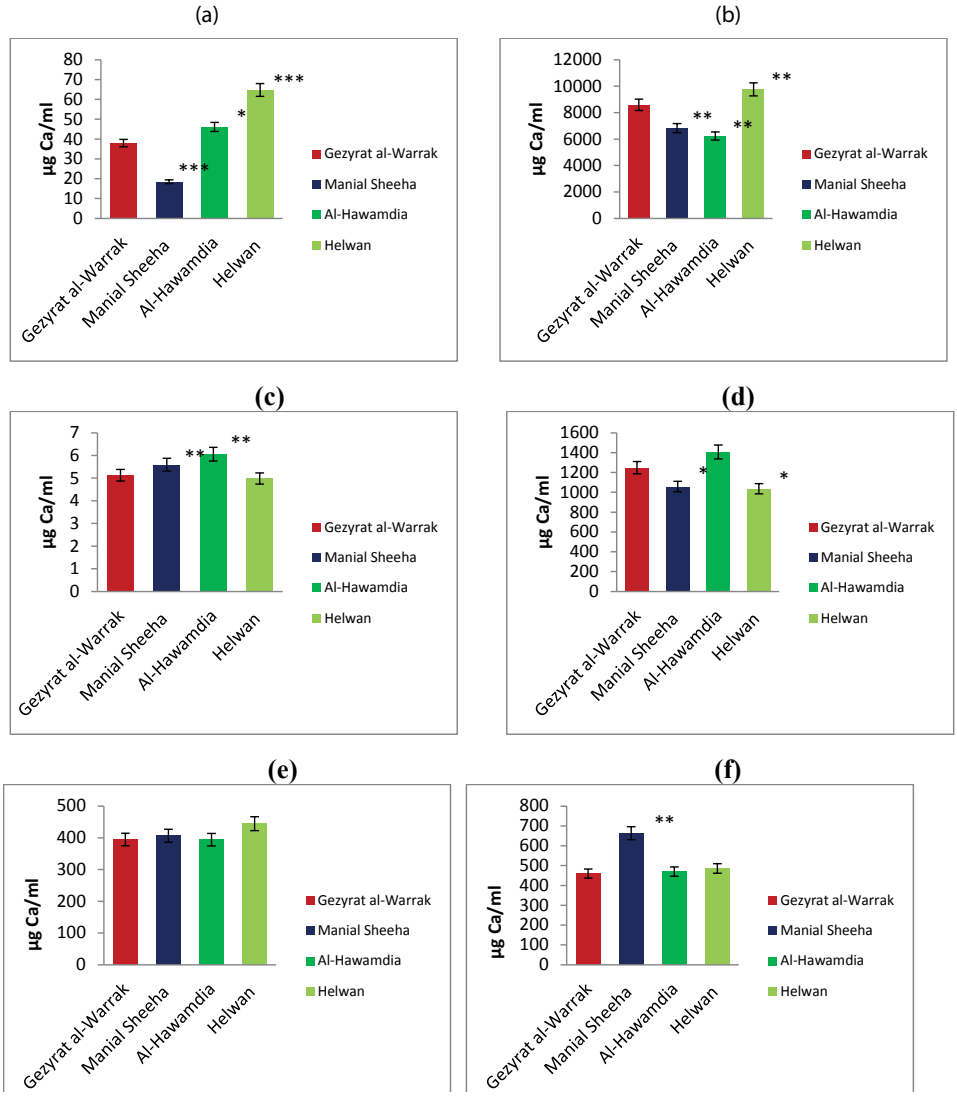

Figure 4: Calcium concentration expressed as µg Ca/ml in water (a), µg Ca/g in wet weight sediment (b) and fresh tissues : exoskeleton (c), gills (d), hepatopancreas (e) and muscles (f) of *P. clarkii* at the different sites of the investigation; levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	muscles
Gezyrat al-Warrak (Site I)	Mean	1.351	5470	14.10	7.994	37.8	14.19
	S.D.	0.068	325.0	0.866	0.855	5.064	0.855
Manial Sheeha (Site II)	Mean	2.343**	4597**	11.19**	7.099	71.27***	11.98**
	S.D.	0.061	346.8	1.362	0.964	6.168	1.164
Al-Hawamdia (Site III)	Mean	1.817**	1080***	10.91**	6.187	45.38	12.68
	S.D.	0.087	208.2	1.278	0.404	5.046	0.768
Helwan (Site IV)	Mean	2.030**	3144***	13.69	14.15***	50.73**	12.27*
	S.D.	0.046	264.4	1.478	1.844	5.180	1.045

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated ad compared with site I as a control site (*P<0.05, **P<0.01 and *** P<0.001)

Table 4: Concentration of zinc (Zn)expressed as µg Zn/ml in water, µg Zn/g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkii*.

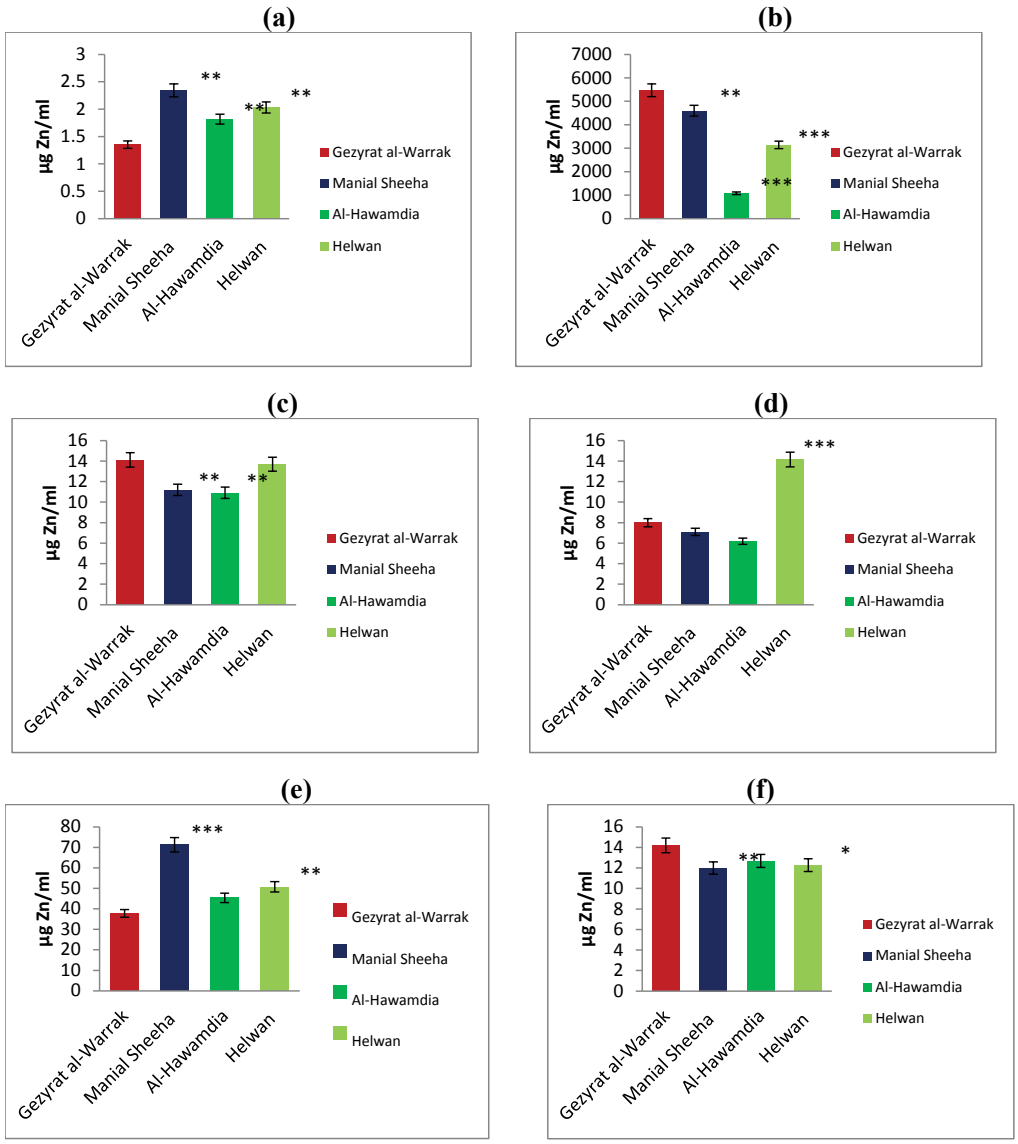

Figure 5: Zinc concentration expressed as µg Zn/ml in water(a), µg Zn/g in wet weight sediment (b) and fresh tissues: exoskeleton (c), gills, hepatopancreas (e) and muscles (f) of *P. clarkii* at the different sites of the investigation; levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	muscles
Gezyrat al-Warrak (Site I)	**Mean**	8.750	3745.0	0.202	0.273	0.306	0.239
	S.D.	1.125	502.62	0.026	0.029	0.048	0.037
Manial Sheeha (Site II)	**Mean**	7.063***	6598.0**	0.257*	0.224	0.183***	0.227
	S.D.	0.092	984.31	0.027	0.027	0.036	0.023
Al-Hawamdia (Site III)	**Mean**	3.861***	6662.0**	0.192	0.156*	0.151***	0.208
	S.D.	0.082	872.60	0.043	0.024	0.028	0.059
Helwan (Site IV)	**Mean**	3.064***	10570***	0.328**	0.804***	0.310	0.226
	S.D.	0.063	1369.0	0.022	0.114	0.041	0.032

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (*P<0.05, **P<0.01 and *** P<0.001).

Table 5: Concentration of cadmium(Cd) expressed as µg Cd/ml in water, µg Cd/g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkia.*

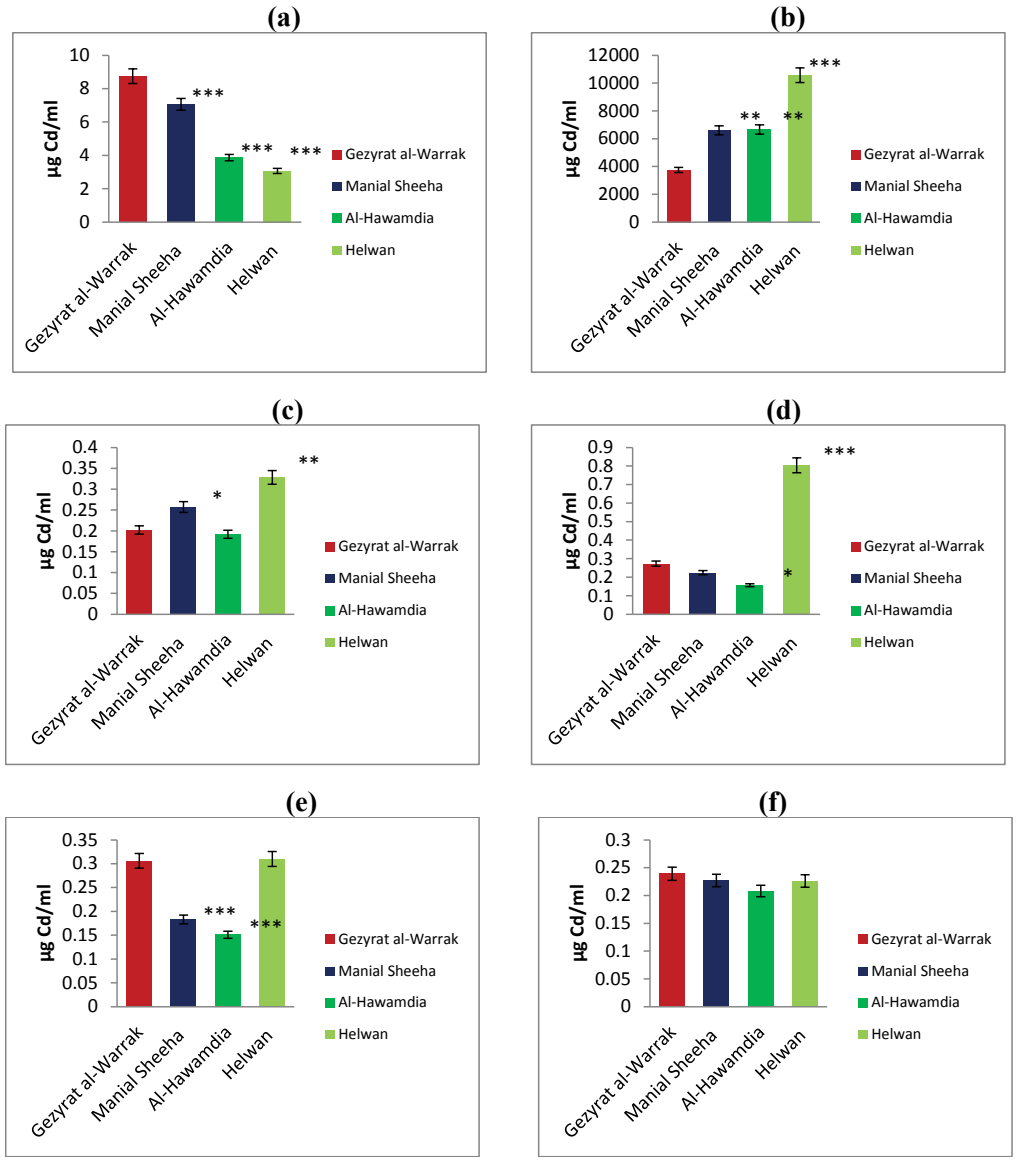

Figure 6: Cadmium concentration expressed as µg Cd/ml in water(a), µg Cd/g in wet weight sediment (b) and fresh tissues in exoskeleton (c), gills, hepatopancreas (e) and muscles (f) of *P. clarkii* at the defferent site of the investigation levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	muscles
Gezyrat Al-Warrak	Mean	11.07	6580	0.921	0.911	0.359	0.541
	S.D.	1.982	289.4	0.128	0.141	0.090	0.090
Manial Sheeha (Site II)	Mean	27.24***	6679	0.502***	0.646**	0.212**	0.546
	S.D.	2.915	312.8	0.039	0.202	0.066	0.110
Al-Hawamdia (Site III)	Mean	44.29***	1217***	0.0639***	0.936	0.648***	0.910***
	S.D.	3.821	152.4	0.130	0.134	0.073	0.112
Helwan (Site IV)	Mean	35.29***	1688***	1.263***	1.022	0.322	0.554
	S.D.	3.926	135.5	1.533	0.174	0.069	0.089

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (P<0.05*, **P<0.01 and *** P<0.001)

Table 6: Concentration of lead (Pb) expressed as µg Pb/ml in water, µg Pb /g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkii*.

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	muscles
Gezyrat al-Warrak(Site I)	Mean	0.009	368.4	53.14	19.88	59.17	2.510
	S.D.	0.0003	34.51	4.318	2.622	1.245	0.426
Manial Sheeha (Site II)	Mean	0.013**	142.1***	70.35***	15.92*	21.70***	1.563**
	S.D.	0.002	19.91	5.714	2.788	1.648	0.173
Al-Hawamdia (Site III)	Mean	0.012**	338.9*	60.31	18.48	29.76***	2.282
	S.D.	0.003	38.43	9.326	2.506	3.566	0.557
Helwan (Site IV)	Mean	0.013**	408.9***	65.36*	24.10*	20.58***	1.255***
	S.D.	0.004	41.52	4.619	4.105	2.708	0.282

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (*P< 0.05, **P<0.01 and *** P<0.001).

Table 7: Concentration of manganese (Mn) expressed as µg Mn/ml in water, µg Mn /g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkii*.

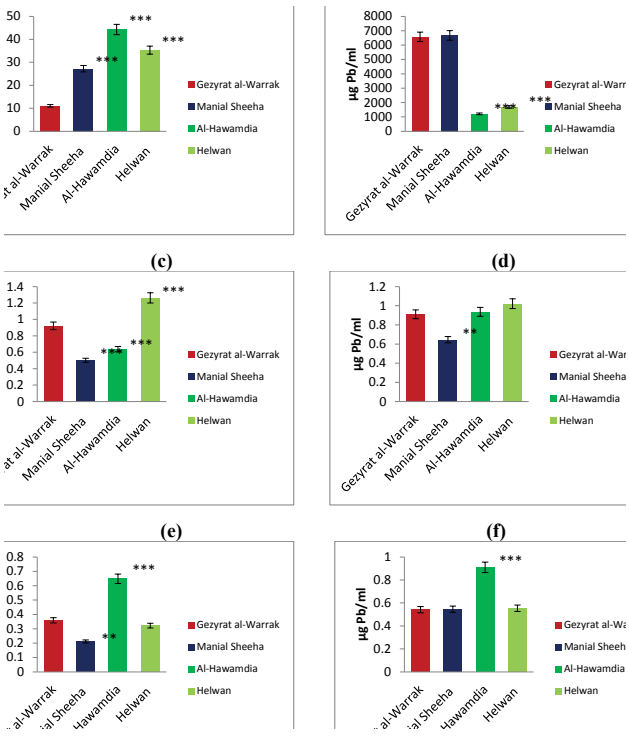

Figure 7: Lead concentration expressed as µg Pb/ml in water(a), µg Pb/g in wet weight sediment (b) and fresh tissues: in exoskeleton (c), gills, hepatopancreas (e) and muscles (f) of *P. clarkia* at the different sites of the investigation levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

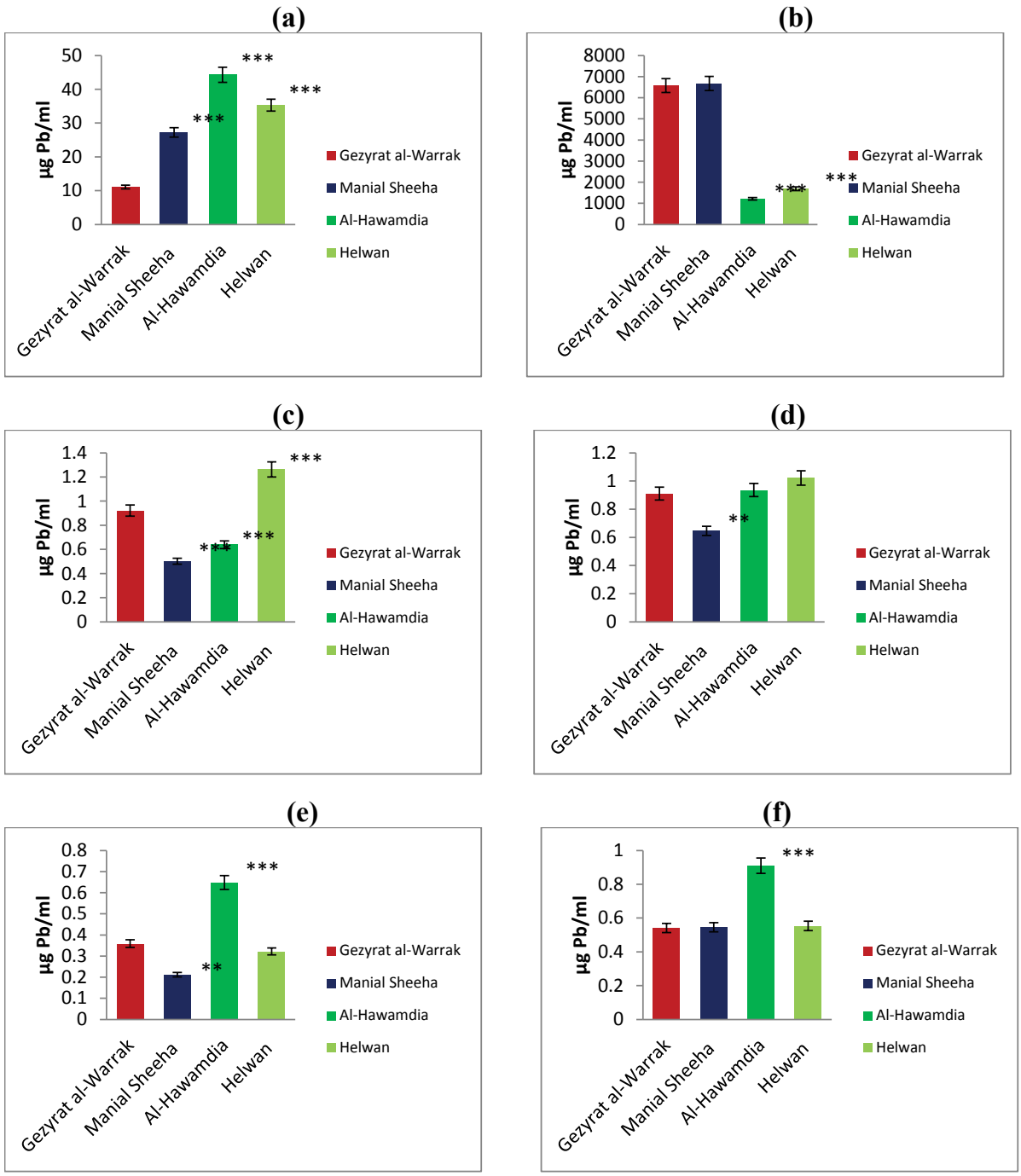

Figure 8: Manganese concentration expressed as µg Mn/ml for water(a), µg Mn/ml in wet weight sediment (b) and fresh tissues; exoskeleton (c), gills, hepatopancreas (e) and muscles (f) of *P. clarkii* in the different sites of the investigation levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

Cu, Cd, Pb and Mn were within the permissible limits, according to USEPA [14] and Khallaf et al. [15]. Whereas, Fe and Zn concentrations were higher than the allowable levels. The presence of Fe and Zn in high concentrations, in the River Nile water, could be attributed to the illegal discharges of industrial wastes and household materials and the considerable amounts of Zn leached from the protective plates of boats that contain the active Zn [16-18]. The concentrations of Cd, Cu, Fe, Pb, Mn and Zn, in the current study, show higher values than those reported by previous authors, while, Cu and Mn were whithin the same ranges [19-23]. These authors arrived to the conclusion that the differential concentrations of metals, in the River Nile water, depend on the seasonal variations and the types of discharges, which is an acceptable explanation for the difference in metal accumulation in the tissues of crayfish.

The River Nile sediment showed more or less different patterns of metal concentrations which were many folds higher than their values in the overlaying water. When compared to results obtained from other aquatic environment in Egypt [24], the present data show higher

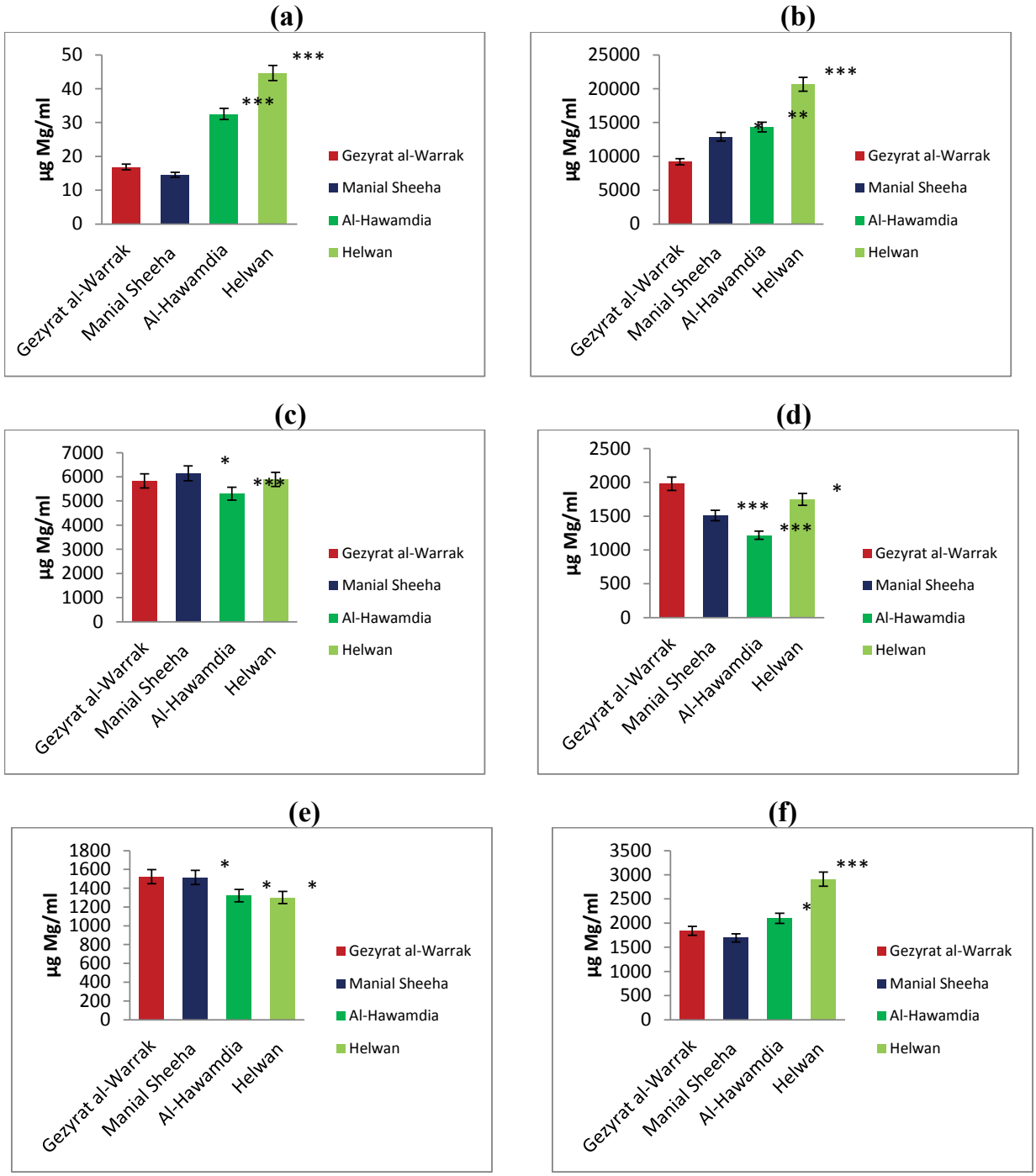

Figure 9: Magnesium concentration expressed as µg Mg/ml in water (a), µg Mg/g in wet weight sediment (b) and fresh tissues: in exoskeleton (c), gills, hepatopancreas (e) and muscles (f) of *P. clarkii* at the different sites of the investigation; levels of significance are *P<0.05, **P<0.01 and ***P<0.001.

concentrations of Fe, Cu, Zn, Mn and Cd, in the sediment of the studied sites. It assumed that the broken down organic matter exists as humus in the sediment which has high colloidal content and a very high absorption capacity. This explains the high concentration of metals recorded in the sediment, herein. Also, Freedman [25] postulated that not only the effect of industrial wastes but also natural mechanism may affect high accumulation of metals in sediments.

Many studies estimated metal accumulation in crayfish tissues [8,9,26-33]. Generally, crustaceans accumulate some metals in direct proportion to the increase in the bioavailability from water and food [34]. However, *P. clarkii*, in the present study, accumulated heavy metals in its tissues regardless their abundance in the ambient water and/or sediment. Fe concentration was significantly higher in the exoskeleton, gills and hepatopancreas of *P. clarkii* than in water, at all sites of the investigation. Also, Kouba et al. [30] showed that even under relatively

Area		Water (n=5)	Sediment (n=5)	Animal Tissue (n=6)			
				Exoskeleton	Gills	Hepatopancreas	muscles
Gezyrat al-Warrak (Site I)	Mean	16.86	9203.0	5827	1980	1523	1842
	S.D.	3.116	1969	129.7	132.7	93.99	163.7
Manial Sheeha (Site II)	Mean	14.54	12900*	6146*	1509***	1515	1695
	S.D.	30.061	1682	166.1	164.3	145.4	190.8
Al-Hawamdia (Site III)	Mean	32.55***	14340**	5304***	1217***	1320*	2100*
	S.D.	3.821	1835	236.5	66.93	259.2	66.93
Helwan (Site IV)	Mean	44.62***	20650***	5890	1747*	1301*	2909***
	S.D.	3.082	2136	100.8	183.4	69.30	108.1

Note: Data expressed as mean ± standard deviation (S.D.). All levels of significance are calculated as compared with site I as a control site (*P< 0.05, **P<0.01 and *** P<0.001).

Table 8: Concentration of magnesium (Mg) expressed as µg Mg/ml in water, µg Mg /g in wet weight sediment and fresh tissues (exoskeleton, gills, hepatopancreas and muscles) of *Procambarus clarkia*.

low concentration in the water, Cd can accumulate in high concentration in some tissues, especially the hepatopancreas. Similarly, Svobodova et al. [31] found a negative correlation between the concentration of Fe in water and in crayfish tissues. Ca in the tissues of the crayfish showed also higher values than its presence in water and sediment. *P. clarkii* showed, in this investigation, a high capacity for Cu accumulation in the exoskeleton. Similarly, it had found that the crayfish has the ability to accumulate Cu in its exoskeleton in considerable amount. The accumulation of Cu in great concentration in the exoskeleton of the crayfish could have a survival value as possible elimination mechanism through molting. Also, Tunca et al. [8] evaluated the relative abundance of Cu, Cd and Pb among other metals in the exoskeleton, gills, hepatopancreas and muscles of the crayfish Astacus leptodactylus and found considerable differences in metal concentration between male and female crayfish.

The concentrations of the detected metals in the exoskeleton of *P. clarkii* showed variable patterns, according to the studied site. The highest concentrations of Mg, Fe and Mn, in the exoskeleton, were observed at site II, whereas Zn and Cu were most accumulated in the exoskeleton, at site I. Ca accumulation recorded high value in the exoskeleton, at site III, whereas, Pb and Cd showed elevated concentrations at site IV. Dissolved Mn is in direct contact with the exoskeleton of the crayfish and its concentration was higher in the exoskeleton than in water due to its incorporation into the calcium carbonate structure, at the post molt stage [34].

Gills of the crayfish are in contact to the external medium and are responsible for metal transfer to organism. This could explain the high metal concentration, recorded herein, in this organ. Meyer et al. [35] has reported also that the gills were the principal site for Cd accumulation, while Pb was concentrated in high level in the exoskeleton and hepatopancreas of the crayfish and the muscles exhibited very low Pb value. Also, Nott [36] reported the highest concentration of Cu in the gills of marine crustaceans and concluded that gills readily absorb it and transport it to the other organs via haemolymph. Furtheremore that the accumulation of Cu in *P. clarkii* was higher in gills of crayfish than in exoskeleton and muscles and attributed this to the process of filtration of water against the gills and taking up the metals through the body surface. Svobodora et al. [32] found that metal accumulation in the gills of crayfish from a polluted locality was significantly higher compared to the reference site, a less polluted locality. Naghsbandi et al. [28] has recorded the highest Fe concentration in gills of crayfish of the same polluted locality. In this respect, Anderson has found also that gills were the most important site for Pb accumulation, when he exposed the crayfish to several concentrations of the metal. *P. clarkii* has a high capacity for Pb accumulation and gills are the most important tissue for Pb accumulation [37,38]. It was found, herein, that Pb accumulation in the gills was in considerable amount as compared to other organs. On the other hand, Svobodora et al. [32] found that the difference in individual metals in muscles, were not as marked as for gills. They reported different patterns in the essential elements Cu and Zn and found that there were no differences between these metals in gills of polluted site and the reference locality. This could be associated with the rapid depuration of these metals [30]. High concentration of metals in water leads to bioaccumulation, decreasing amounts of metals in water ecosystem which can cause depuration of the accumulated metals from tissues. Zn, Cu, Cd, Pb, Ca, Mn Mg and other metals can be depurated in few weeks as suggested by Guner [39] and Kouba et al. [30].

The hepatopancreas plays an important role in heavy metal metabolism and contributes to their detoxification [40]. Kuklina et al. [9] determined the accumulation of Cu, Zn, Cd and Zn in crayfish tissues from three drinking reservoirs in Czech Republic and reported that the hepatopancreas was the principal accumulating organ for most of the metals. Similarly, Kouba et al. [30] found that Cu, Zn, Pb and Cd accumulated mainly in the hepatopancreas. In the current study, the hepatopancreas of the crayfish was found to be a major site for Fe, Cu, Zn, Pb and Mg accumulation in *P. clarkii* tissues.

The lowest concentrations of metals were found in the crayfish muscles in comparison to the exoskeleton, gills and hepatopancreas, except for Ca and Mg, which are considered as essential elements for animal growth and wellbeing. Anderson [41] has reported no metal accumulation in the muscles of the crayfish after placing it for 7 days, in water receiving petroleum – laden effluents. The results of the selected metals in *P. clarkii* muscles were lower than the international permissible level [12]. Therefore, no significant health hazard would result from the consumption of *P. clarkii*, collected from the sites of investigation. However, further investigations are needed to evaluate the nutritional value of this animal.

Macroinvertebrates are frequently suggested as bioindicator for monitoring programs [9,26,42]. *P. clarkii* has been proposed for use as a bioindicator of heavy metals due to its ability to accumulate these environmental pollutants [30,43]. The analysis of trace metals in the various organs of *P. clarkii*, herein, might be useful as bioindicator for metal pollution in the freshwater systems, due to their propensity to rapidly accumulate them. Furthermore, concentration of metals in water leads to bioaccumulation, decreasing amounts of metals in water ecosystem which can cause depuration of the accumulated metals. Guner [39] and Kouba et al. [30] reported that Zn, Cu and Cd can be depurated in a few weeks

Conclusion

P. clarkii collected from the studied sites can be consumed safely by humans as there were no sufficient accumulation of metals in their muscles, as metal concentrations were in the permissible levels for metals in food. *P. clarkii* can be used also as bioindicator of heavy metals in freshwaters ecosystems. Moreover, accumulated metals in the crayfish tissues can cause depuration of accumulated metals by decreasing their amounts in water [44-47].

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