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Assesment of Some Heavy Metals Concentrations in Water Sediment and Ovigerous Female Crayfish *Procambarus clarkii* from Gharbia Drains, Egypt

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Abstract: The present study aimed to assess the concentration of some heavy metals (Pb, Cd, Cu, Co and Zn) in water, sediment and ovarian tissue and egg sac in crayfish *Procambarus clarkii* from El-Kased drain at Eshnawi (site I, Sewage drainage water) and Ezbat Toukh drain (site II, agriculture drainage water), in Gharbia Governorate, Egypt during March 2005, In addition, biochemical and histochemical studies on the investigated eggs from the two sites of collection were carried out . Analyses of sediments and tissues revealed heavy contamination by Zn, Cu and Co. Lead and Cadmium were not detected in spite of their presence in water. The data of toxicological analysis revealed that the levels of accumulation of heavy metals in tissues, are arranged in the following decreasing order: Cu > Zn > Co. The percentage of protein in eggs from the two sites is slightly different. It was concluded that the investigated animal may have the ability to regulate the excess percent of heavy metals. Therefore there were no pathological changes on the histochemical structure of the eggs.

Key words: Heavy metals · Water · Sediment · Freshwater crayfish · Decapoda · Crustacea · Biochemical · Histochemical studies

INTRODUCTION

Pollution of aquatic ecosystem by heavy metals is a widespread problem [1 -3]. Sewage effluents, industrial discharges, autoemissions, dredging activities and garbage dumps are some of the major sources of metal pollution of aquatic ecosystem.

Metals, after entering the water, may precipitate or adsorb on solid surfaces, remain soluble or suspended in water or may be taken up by fauna and flora [4,5]. Some heavy metals in trace concentrations are normal constituents of aquatic organisms, but at higher levels they are potentially toxic and may severely interfere with the ecology of the aquatic environment [6]. The toxicity of heavy metals is due to their ability to be concentrated in the organs of aquatic organisms [7,8], thus posing a direct threat to both aquatic biota and man [9].

Freshwater crayfish play an important role in the trophic chain of benthic communities in lakes and rivers and contribute to the regulation of freshwater ecosystems[10,11]. In polluted environment , crayfish are able to accumulate a considerable amount of heavy metals in their tissues and to excrete some of them [12,13]. The concentrations of heavy metals in the crayfish body are regulated by metal-binding proteins, hepatic metallothioneins to a constant level until metal bioavailability exceeds the higher threshold, when the regulation breaks down and net accumulation begins [14].

Crayfish can be used to monitor the aquatic environments for heavy metal pollution because they are solitary bottom dwellers, which keep much of their bodies in contact with surrounding objects [15,16]. Many studies have been undertaken on the accumulation of heavy metals in the freshwater crayfish, in the carapace, muscles, digestive gland, gills and brain[17-22]. Also, the effect of heavy metals on reproduction and life history stages of some crustacean have been studied [23,24]. Some studies showed some pathological and biochemical disturbances in aquatic organisms according to the pollution of water [25,26].

The present study aimed to: 1-assess the level of some heavy metal concentrations (Pb, Cu, Cd, Zn and Co) in two sites polluted by different sources, sewage outfall (site I) and agriculture drain (site II). 2- show if these level

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of metal concentrations can induce pathological and biochemical changes in ovary and eggs or not.

MATERIALS AND METHODS

Site of Collection: Water, surface sediments and ovigerous females of crayfish *Procambarus clarkii* were collected from El-Kased drain at Eshnawi (site I, Sewage drainage water) and Ezbat Toukh drain (site II, agriculture drainage water), in Gharbia Governorate, Egypt during March 2005. Soon after collection the water samples were filtered on 0.45 μm Millipore filter and then preserved in plastic bottles by the addition of a few drops of nitric acid until measurements. Sediments from the investigated sites were dried at room temperature then kept in plastic bags until analysis. Ovigerous females crayfish with total length of 9 to 10 cm and weight of 16 to 17 g in were taken from various holes of the two sites (Fig. 1a,b). Ovary was dissected and egg sac (Fig. 1a) was removed from the abdominal appendages of ten specimens from each site and preserved separately until analyzed.

Histological and Histochemical Analysis: Egg sac of ovigerous females from each site was removed and fixed in Bouin's fluid for 48 hours. Then, they were dehydrated in ascending series of ethyl alcohol, cleared in terpineol and embedded in paraffin wax. The blocks were sectioned serially at 4-6 μm. Sections were stained with four standard methods: (1) haematoxylin and eosin to study gross qualitative changes of the egg from each site, (2) bromophenol blue to quantify proteins, (3) sudan black to quantify lipids and (4) PAS to quantify carbohydrates with the egg from each site. Slides were examined with light microscope and images were captured using a digital image system (Olympus CX 31)connected to a computer. The diameter range of eggs was determined by using an objective micrometer.

Biochemical Analysis: Moisture was determined by obtaining wet and dry weight to the nearest 0.01 g, following drying overnight at 80°C [27]. Moisture percentage was then calculated by dividing dry weight by wet weight and multiplying by 100.

Protein was determined by the kjeldhal method, while fat was determined by Sokselet method [28].

Heavy Metals Analysis

For Water: The concentration of heavy metals in water samples were extracted by using the APDC-MIBK procedure [29]. The pH of investigated water samples were adjusted to about pH 3-4, add 5ml ammonium pyrrolidine dithiocarbamate (APCD) solution and shake to mix 50 ml of methyl isobutyle keton (MIBK) was added and shaked for 30 s. The extracted organic layer was aspirated directly to the atomic absorption spectrophotometer

For Sediments: Sediment samples 0.5 g were digested in Teflon vessels for 2 hrs with a mixture of concentrated acids (HNO_3, $HClO_4$ and HF) according to the method described by Origioni and Aston [30].

For Soft Tissue: Heavy metals were measured in ovary and egg tissues according to Dybern [31]. The ovary and eggs of ovigerous females crayfish from each site were weighed, then freshly prepared at 1 : 1 v/v hydrogen peroxide/conc. HNO_3 was added to each in glass tubes for about 2 hrs. The tubes were placed in a sand path at about 160°C till complete digestion. After that, the solubilized samples have been diluted to 25 ml double-distilled water. The prepared aqueous solutions were analyzed by using atomic absorption spectrophotometer to determine the selected heavy metals (Pb, Cu, Cd, Zn and Co).

Element concentration in the sample (Mg/g wet weight) =

$$\frac{\text{Con. of element in solution X volume of solution}}{\text{weight of the sample}}$$

Statistical Analysis: The values of different concentration factors were calculated from the following equation:

$$\text{C.F.} = \frac{\text{Con. of heavy metals in tissue}}{\text{Conc. of heavy metals in water}}$$

Data were reported as Mean±SE. Analysis of variance using least significant difference as comparative of means was carried out [32]. The level of significance was presented at $P < 0.05$.

RESULTS

Histological and Histochemical Investigation: The egg of the herein species has on ovate shape (Fig. 2) and large size with diameter ranges between 1.6 and 1.8 mm. The egg contents are readily stratified into three zones: the first zone is the outer vitelline membrane. This membrane,

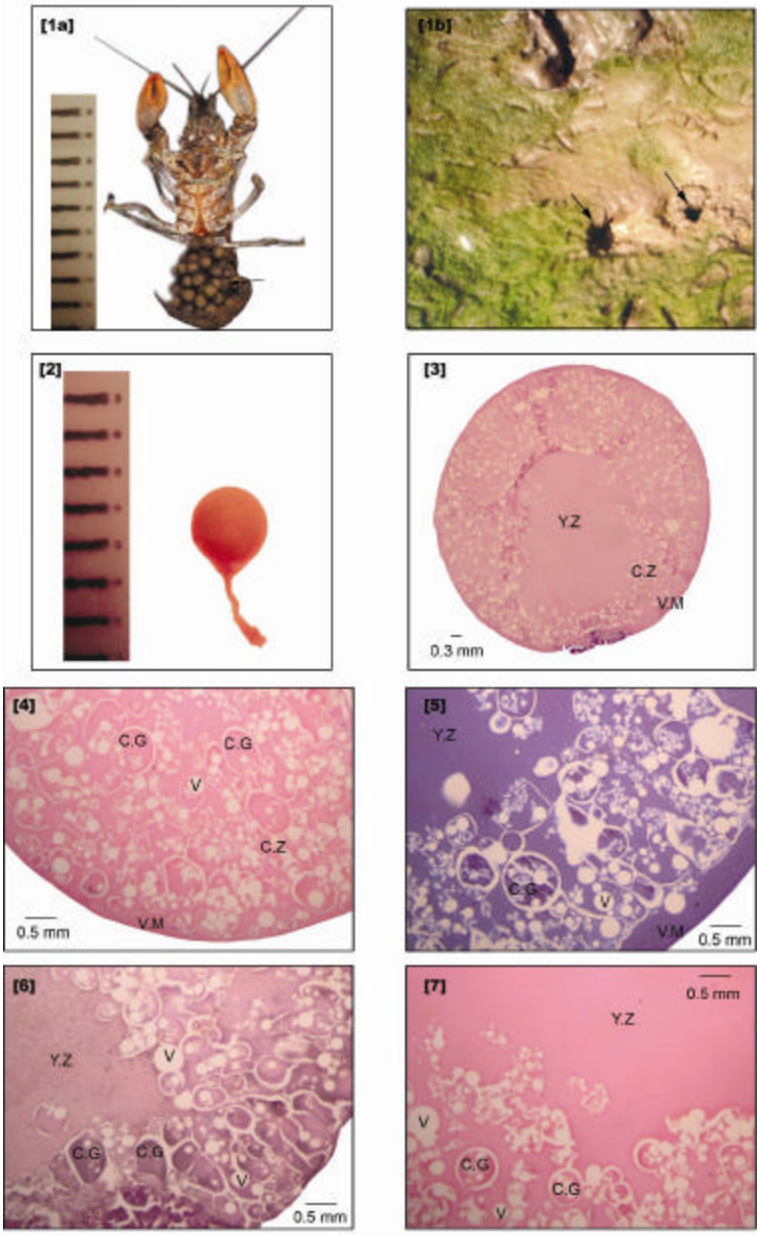

Fig. 1: (a): Ovigerous female of crayfish, *Procambarus clarkii* showing the egg sac carried on abdominal appendages
(b): Shows the holes (arrows) in which the specimens are embedded in the sites of collection

Fig. 2: Shows that the egg has an ovate shape

Fig. 3: Light photograph of egg stained with H & E showing the three zones. vitelline membrane (V.M), cortical zone (C.Z) and yolk zone (Y.Z). bar = 0.3 mm

Fig. 4: Light photograph of egg stained with H & E showing the alveolar shape of cortical zone. C.G., cortical granule; V., vacuole; V.M., vitelline membrane. Bar = 0.5 mm

Fig. 5: Light photograph of egg stained positively with bromophenol blue. C.G, cortical granule; V, vacuole; V.M, vitelline membrane; Y.Z, yolk zone. Bar = 0.5 mm

Fig. 6: Light photograph of egg stained positively with Sudan black B. CG, cortical granule; V, vacuole; Y.Z, yolk zone. Bar = 0.5 mm

Fig. 7: Light photograph of egg stained positively with PAS. C.G, cortical granule; V, vacuole; Y.Z, yolk zone. Bar = 0.5 mm

which appears homogenous and structureless, is very thick, enveloped and protected the egg (Fig. 3). The second zone is the cortical zone, which made up of coarse alveolar ectoplasm. The cytoplasm is filled with granules of different constitution and sizes, some are large cortical granules and other unidentified granules. The cortical granules are usually located under the plasma membrane, some of them are displaced inwardly in ectoplasm by movement during preparation. Each granule is limited by a smooth membrane. The chemical nature of the substance filling the granule, is that of lipoprotein because it stains positively with bromophoenol blue and Sudan black B. Also, the ectoplasm shows numerous vacuoles of different shapes and sizes giving this zone its alveolar appearance (Fig. 4- 6).

The third and inner zone is the yolky zone (endoplasm). The endoplasm is mostly made up of greatly fine yolk granules (Fig. 3). The yolk, which is the major source of energy for embryonic metabolism, mostly made up of fat and proteins. The abundance of substances is indicated by the positive reactions with their specific stains (Fig. 5, 6). Also, polysaccharides appear to be evenly distributed in the cytoplasm (Fig. 7).

Average diameter and histochemical structure of eggs taken from two studied sites I & II didn't vary significantly by location, indicating that egg sampling need not consider locational bias.

Biochemical Studies of Egg Sac: Table 1 presents the percent organic composition of egg sac in relation to the locality. Egg sac from site II demonstrates a slight decrease in weight than that from site I. There is no marked difference exists between the eggs from two sites with respect to protein. They have a relatively high protein content. In contrast, there is a noticed decrease of lipid content value in site I than that of site II. Perhaps the sort of pollution effects on the lipid content of egg sac.

Heavy Metals Accumulation: Mean metal concentrations of five heavy metals in water, sediment, ovarian tissue and egg sacs, in the two sites I & II are summarized in Table 2 and Fig. 8. Concentration of metals (Pb, Cu, Cd, Zn and Co) in sediment and stream water measures as the environmental baseline conditions.

The results presented in Table 2 indicate that all the metals, except Pb and Cd are accumulated to a greater or lesser extent by the sediment and investigated tissues. However, Pb and Cd were not detected at all of them. In general, the tissue accumulation of nonessential metals (Pb, Cd) reflected environmental concentrations (Fig. 8).

Table 1: Chemical composition of egg sac from the two sites I and II. Values for protein and lipid content expressed as percent dry weight

Site/ chemical composition	Site I	Site II
Weight of egg sac	1.7 g	1.5 g
Protein	19.1%	19.3%
Fat	13.3%	15.17%
Moisture	56.22%	64.95%

Table 2: Mean and standard error (SE) of heavy metals concentration in the ovary and egg of *Procambarus clarkii* (μg/l) and sediments (μg/g) at two sites of collection

		Sites	
Metals		Site I Mean±SE	Site II Mean±SE
Lead (Pb)	Water	12.400±0.1	8.300±0.15*
	Sediment	ND	ND
	Ovary	ND	ND
	Egg	ND	ND
Cupper (Cu)	Water	77.500±0.15	47.200±0.13*
	Sediment	17.500±0.15	18.217±0.1
	Ovary	46.750±0.04	121.290±0.13*
	Egg	62.340±0.05	46.030±0.o88*
Cadmium (Cd)	Water	1.700±0.1	1.200±0.1*
	Sediment	ND	ND
	Ovary	ND	ND
	Egg	ND	ND
Zinc (Zn)	Water	112.100±0.05	125.100±0.05*
	Sediment	35.130±0.09	40.053±0.052*
	Ovary	13.380±0.22	28.000±0.17*
	Egg	20.220±0.15	14.320±0.075*
Cobalt (Co)	Water	2.100±0.05	2.633±0.07*
	Sediment	8.830±0.12	12.720±0.07*
	Ovary	0.450±0.04	1.660±0.06*
	Egg	1.260±0.02	0.630±0.009*

* Significant at P-value <0.05

The Sediment and Water Data: The results provide evidence for the relatively high metal concentration (Zn, Co) in site II as compared with those from site I (Table 2). On the other hand, the mean concentrations of Pb, Cu and Cd, especially in water, are higher in site I than those of site II. Zn in the water was the highest of all metals ,while Cd is the lowest. Cd and Co levels in the water didn't differ significantly between the two sites. The sediment data indicated maximum concentration (40.053 μg/g) of Zn at site II, while Co shows the minimum concentration (8.830 μg/g) at site I. An overall trend existed of increasing metal accumulation of sediment among site II while site I normally showed lower metal concentrations.

Tissue Metal Concentration: A closer examination of the data in Table 2 shows that the ovarian tissue and egg sac accumulated heavy metals generally in the following sequence: Cu > Zn > Co > Pb and Cd. Average values for accumulated metals were exceedingly higher in site II than in the site I in the case of ovary. Concerning egg sac, the average concentrations of metals are higher in site I

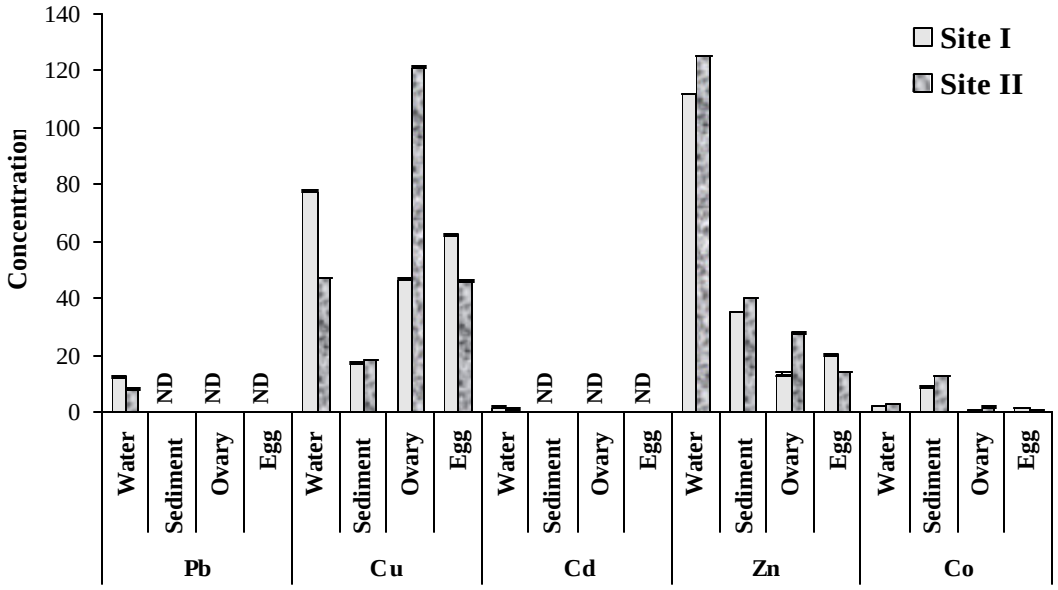

Fig. 8: Concentration of heavy metals (Pb – Cu – Cd – Zn – Co) in water (µg/l); sediment (µg/g), ovary and eggs of *Procambarus clarkii* from two sites of collection

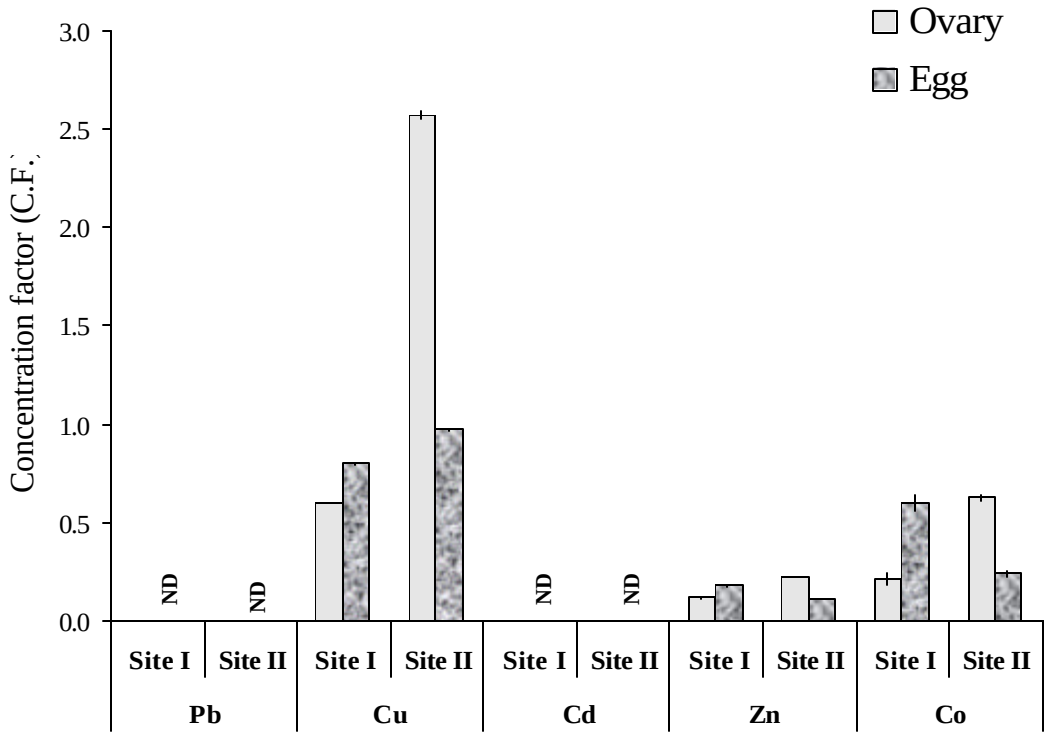

Fig. 9: Concentration factor (C.F) of the various metals for the ovary and eggs of *Procambarus clarkii* in two sites of collection

Table 3: Concentration factor (C.F) of the various metals for the ovary and egg *Procambarus clarkii* from two sites collection

		Organs	
		Ovary	Egg
Metals	Sites	Mean±SE	Mean±SE
Pb	Site I	ND	ND
	Site II	ND	ND
Cu	Site I	0.603±0.002	0.804±0.002*
	Site II	2.570±0.009	0.975±0.0045*
Cd	Site I	ND	ND
	Site II	ND	ND
Zn	Site I	0.119±0.002	0.180±0.0015*
	Site II	0.224±0.0015	0.114±0.001*
Co	Site I	0.214±0.015	0.601±0.02*
	Site II	0.630±0.008	0.240±0.01*

* Significant at P-value <0.05

comparing with those in site II. A comparison of metal concentrations (Fig. 8) indicated that Cu was accumulated more as compared with other metals. The levels of Cu are found to be highest in the ovary (121.29 μg/ g) and egg sac (62.34 μg/g) at the site II and site I , respectively. A comparison between ovary and egg sac in site I shows the increase in metals in egg indicating that the egg membrane actively absorbs metals. While in site II, the ovarian tissues can accumulate higher concentration of metals than the egg sac.

The concentration factor (C.F.) of metals in the investigated tissues of the animal are given in Table 3 and Fig. 9. The C.F. of metals, accumulated in both ovary and egg sac in the two sites I and II, was in the same following order: Cu > Co > Zn. The ovary revealed the highest C.F. of Cu in site II while the lowest C.F. was denoted of Zn in the egg sac at site I.

DISCUSSION

Benthic invertebrates, crayfish among them, are in direct contact with heavy metals both of natural and anthropogenic origin. The discharge of metals by agriculture or sewage drainage represents a serious water pollution problem due to the toxic properties of these elements and their adverse effects on water quality.

Analyses of sediment and water samples revealed large amounts of metals suggesting that metal bioaccumulation in tissues of the herein species might result from interactions of the organism with the contact environment. The obtained results in the present investigation indicate that the concentration of metals in the sediment of the two sites I & II, reveal different patterns of concentrations according to their abundance in water except for only Pb and Cd. This correlation come in accordance with that reported by Heiba [20], that the chemical composition of the sediment depends on the metals in water. Also, it was reported that the sediment acts as a reservoir for all the contaminates and dead organic matter descending from the ecosystem above[33]. Abdel-Baky *et al.* [34] found a correlation between the concentration of heavy metals and the abundance of organic matter. All these results explain the similarity in sequences of element concentrations between water and sediment.

On the other hand, [35] suggested that the sediments are one of the major sinks of trace metals in aquatic environment and the concentrations of heavy metals in the sediment usually exceed those of the overlying water by 3 to 5 orders of magnitude. The present results come in agreement with this suggestion in case of Co only where the concentration of cobalt in sediment exceed than those of water by 4 to 5 times.

The lackage of sediment from both Pb and Cd in herein study, is contradicted with other results obtained from other aquatic environments in Egypt. Zyadah[36] and Zyadah [37] and Abdel-Baky *et al.* [34] reported very high levels of Cd and Pb in different locations of the River Nile at Damietta Governorate and Lake Manzala, respectively.

According to Nagvi *et al.* [38], crayfish under natural conditions are impacted and accumulated heavy metals and can be used for testing metal bioavailability of polluted freshwater. The present data indicate that all the metals, except Pb and Cd, are accumulated to a greater or lesser extent by tissues. Both ovarian tissue and egg sac revealed the following general relationship among metal concentration in the two sites I & II: Cu > Zn > Co > (Pb and Cd) which were not detected at all. Bagatto and Alikhan [39] and Mackeviciene [18] determined a nearly similar relationship among the tissue metal concentration of the crayfishes *Orconectes virilis* and *Astacus astacus*, respectively. The absence of Cd from tissues is in agreement with the results of Sadiq *et al.* [2]. They recorded that nothing is known regarding the accumulation of Cd in Crab.

The highest accumulation level of Cu and Zn found in the ovary and egg sac reflect the storage capacity for heavy metals and also may be related to the metabolic role of each of the metals and to their abundance in the surrounding environment as also conducted by Alcorlo *et al.* [19]. Zinc and Copper constitute essential heavy

metals in crayfish, having many biological effects, but also known as toxicants [18]. They appear to diffuse passively the gradients created by adsorption of membrane surfaces and are bound by blood proteins metallothioneins [12]. Carbonell and Tarazona [40] concluded that different tissues of aquatic animals provide and/or synthesize nonexchangeable binding sites resulting in different accumulation levels.

Due to the fact that these metals (Zn & Cu) are essential, they are also subject to strong regulation being detoxified by metallothioneins [41], eliminated by excretion through faeces or urine and via haemolymph through excretory organs or gills [42]. It could be concluded that the ability of the animal to regulate its tissue burdens of heavy metals and also the lackage of Pb and Cd from the tissue may interpret the absence of hazard or pathological sings of the structure of eggs and the slight difference of chemical composition of the egg sac from the two studied sites.

REFERENCES

1. Forstner, U. and G.T.W. Wittman, 1979. Metal pollution in the aquatic environment. Springer-Verlag. New York.
2. Sadiq, M., T.H. Zaidi, A.U. Hoda and A.A. Mian, 1982. Heavy metal concentrations in shrimp, crab and sediment obtained from AD-Dammam Sewage outfall area. Bull. Environ. Contamination Toxicol., 29: 313- 319.
3. Ober, A.G., M. Gonzalez and I.S. Maria, 1987. Heavy metals in Molluscan, Crustacean and other commercially important Chilean Marine Coastal water species. Bull. Environ. Contamination Toxicol., 38: 534-539.
4. Leland, H.V., 1977. Distribution of solute and particulate trace elements in Souther Lake, Michigan. Proceeding International Conference on Heavy metals in the Environment, II/2,17.
5. Oehme, F.W., 1978. Toxicity of Heavy Metals in the Environment, Part I, Marcel Dekker Inc., New York, pp: 515.
6. Bryan, G.W., 1971. The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proceeding Royal Soc. London, 13: 117: 389-410.
7. Thrower, S.J. and I.J. Eustace, 1973. Heavy metal accumulation in oyster grown in Tasmanian waters. Fd Technology Australian, 25:546-553.
8. Watling, H.R., 1983. Accumulation of seven metals by *Crassostrea gigas, C. margaritacea, Perna perna and Chloromytilus meridionalis.* Bull. Environ. Contamination Toxicol., 30: 213-320.
9. Bryan, G.W., 1976. Heavy metal contamination in the sea. In: Johnstan R (Ed) Marine Pollutian. Academic Press, London, pp: 185-302.
10. Momot, W.T., 1995. Redefining the role of crayfish in aquatic ecosystem. Review in Fisheries Science, No. 3: 33-63.
11. Creed, R.P. and J.M. Reed, 2004. Ecosystem engineering by crayfish in a headwater stream community. J. North American Benthol. Soc., 23:224- 236.
12. Bryan, G.W., 1968. Concentration of zinc and copper in the tissues of decapod crustaceans. J. Marine Biol., 48: 303-321.
13. King, H.M., D.S. Baldwin, C.N. Rees and S. McDonald, 1999. Apparent bioaccumulation of Mn derived from paper- mill effluent by freshwater crayfish *Cherax destructor* in the role of Mn oxidizing bacteria. Sci. Total Environ., 9: 226 (2-3): 61-67.
14. Rainbow, S. and S.L. White, 1989. Comparative strategies of heavy metal accumulation by crustaceans: Zinc, copper and cadmium in decapod, an amphipod and a barnacle. Hydrobiologia, 174: 245-262.
15. Khan, A.T., D.M. Forester and H.W. Mileke, 1995. Heavy metal concentrations in two populations of crayfish. Vet Hum Toxicol., 37: 426-428.
16. Schilderman, P.E.L., E.J.C. Moonen, L.M. Maas, I. Welle and J.C.S. Kleinjans, 1999. Use of crayfish in biomonitoring studies of environemtnal pollution of the River Meuse. Ecotoxicol. Environ. Safety, 44: 241-252.
17. Suedel, B.C., J.H. Rodgers and E. Deaver, 1997. Experimental factors that may affect toxicity of cadmium to freshwater organisms. Arch. Environ. Contamination Toxicol., 33(2): 88-98.
18. Mackeviciene, G., 2002. Bioaccumulation of heavy metals in noble crayfish (*Astacus astacus* L.) tissues under aquaculture conditions. Ekologija, 2: 79-82.
19. Alcorlo, P., M. Otero, M. Crehuet, A. Baltanas and C. Montes, 2006. The use of the red swamp crayfish (*Procambarus clarkii,* Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). Science of the Total Environment, 366: 380-390.

20. Heiba, F.N., 2006. Accumulation of some heavy metals in the brain of the freshwater crayfish *Procambarus clarkii* (Crustacea, Decapoda). J. Egyptian Germany Soc. Zool., 51D: 1-20.
21. Naghshbandi, N., S. Zare, R. Heidari and S. Soleimani Palcheglu, 2007. Bioaccumulation of lead nitrate in freshwater crayfish (*Astacus leptodactylus*) tissues under aquaculture conditions. Pak. J. Biol. Sci., 10(18): 3245-3247.
22. Williams, G., J.M. West and E.T. Snow, 2008. Total arsenic accumulation in Yabbies (*Cherax destructor clark*) exposed to elevated arsenic levels in Victorian gold mining areas. Australia. Environ. Toxicol. Chem., 27: 1332-1342.
23. Rodriguez, E.M., M. Schuldt and L. Romano, 1994.Chronic histopathological effects of parathion on female gonads of *Chasmagnathus granulata* (Decapoda, Brachyura). Food Chem. Toxicol., 32 (9): 811-818.
24. Morsy, A.M.M., 2001. Studies on the freshwater crayfish *Procambarus clarkii* (Cambaridae, Crustacea) recently invading the Egyptian freshwater. Ph.D thesis Faculty of Science, Tanta University,Egypt.
25. Mance, G., 1987. Toxicity of metals to marine life. In: pollution threat of heavy metals in aquatic environments (Kenneth Mellanaby Ed.), Elsevier Applied Science. London, New York., 174-232.
26. Meyer, W., M. Krestschmer, A. Hoffmann and G. Harisch, 1991. Biochemical and histochemical observations on effects of low level heavy metal load (Lead- Cadmium) in different organ systems of the freshwater crayfish *Astacus astacus*. Ecotoxicol. Environ. Safety, 21: 137-156.
27. Huner, J.V., 1995. Ecological observation of red swamp crayfish *Procambarus clarkii* and white river crayfish *Procambarus zonangulus* as regards their cultivation in earthen ponds. Fresfwater Crayfish, 10: 456- 468.
28. A.O.A.C. (Association of Official Analytical Chemists), 1984. Official Methods of Analysis Association of Agricultural Chemists. Washington, D.C. USA.
29. APHA, 1989. AWWA and WPCF standard methods for examination of water and waste water, 17th Edn. APHA, Washington.
30. Origioni, B. and S.R. Aston, 1984. The determination of selected trace metals in marine sediments by flameless flame atomic absorption spectrophotometry. IAEA Monaco Laboratory, International Report.
31. Dybern, B., 1983. Field sampling and preparation of subsamples of aquatic organisms for analysis of metals and organochlaines, FAO, Fisher. Technology, 212: 1-13.
32. Selvin, S., 1996. Statistical Analysis of Epidemiologic Data. 2nd Edn., Oxford, University Press. New York, London, pp: 44-78.
33. Hamed, M.A., 1998. Distribution of trace metals in the River Nile ecosystem Damietta branch between Mansoura city and Damietta Province. J. Egypt Germany Soc. Zool., 27: 399-415.
34. Abdel-Baky, T.E., A.E. Hagras, S.H. Hassan and M.A. Zyadah, 1998. Environmental impact assessment of pollution in lake Manzala. 1. Distribution of some heavy metals in water and sediment. J. Egypt Germany Soc. Zool., 26: 25-38.
35. Szefer, P., F. Paez-Osuna, A.A. Ali, A.C. Ruiz-Fernandes, S.R. Galvan and J. Geldon, 1998. Distribution and association of trace metals in soft tissue and byssus of *Mytelle strigata* and other benthal organisms from Mazatlan harbour, mangrove lagoon of the northest coast of Mexico. Environ. Intl., 24: 16.
36. Zyadah, M., 1996. Occurrence of some heavy metals in two aquaculture systems at Damietta Province. Egyptian Journal of Union Arab Biology, 6: 219-232.
37. Zyadah, M., 1997. A study on levels of some heavy metals in River Nile estuary-Damietta branch, Egypt. J. Egypt Germany Soc. Zool., 23: 149-160.
38. Nagvi, S.M., N. Devalraju and H. Nagvi, 1998. Copper bioaccumulation and depuration by red swamp crayfish, *Procambarus clarkii*. Bull. Environ. Contamination Toxicol., 61: 65-71.
39. Bagatto, G. and M.A. Alikhan, 1987. Copper, cadmium and nickel accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. Bull. Environ. Contamination Toxicol., 38: 540-545.
40. Carbonell, G. and J.V. Tarazona, 1994. Toxicokinetics of Cu. Aquacult. Toxicol., 29: 213-221.
41. Canli, M., R.M. Stagg and G. Rodger, 1997. The induction of metallothionein in tissues of the Norway lobster *Nephrops norvegicus* following exposure to Cd, Cu and Zn: the relationships between metallothionein and the metals. Environ. Pollution, 96(3): 343-350.
42. Arumugtan, M. and M.H. Ravindranath, 1987. Copper toxicity in the crab, *Scylla serrata,* Cu levels in tissues and regulation after exposure to a Cu-rich medium. Bull. Environ. Contamination Toxicol., 39: 708-715.