Concentrations Of Some Heavy Metals In Crayfish (*Procambarus clarkii*) As A Biological Indicator Of Aquatic Pollution

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ABSTRACT

A total number of one hundred and twenty crayfish (*Procambarus clarkii*) and six water samples were collected from natural water sources from three different localities, Zagazig, Sharkia Province, Benha, Qalyubia Province and Meetghamr, Dakahlia Province for detection and determination of zinc (Zn), lead (Pb), copper (Cu), silver (Ag), nickel (Ni) and mercury (Hg) by using Atomic Absorption Spectrophotometer.

The results of water analysis revealed that the concentration of lead was significantly higher in Zagazig followed by Meetghamr and then Benha ($p \leq 0.05$). Also the concentration of mercury was significantly higher in Zagazig and Benha than Meetghamr ($p \leq 0.05$), while the other elements showed non significant differences between the examined localities. On the other hand, the results of the crayfish analysis revealed that the concentration of copper was significantly higher in crayfish obtained from Benha and Meetghamr than those obtained from Zagazig ($p \leq 0.05$), while the other elements showed non significant differences between the crayfish obtained from the examined localities.

It was found that some heavy metals were above the permissible limits in water and crayfish samples in the examined localities. The obtained results revealed positive correlation ($p \leq 0.05$) between different heavy metals in water samples and the same heavy metal residues in crayfish samples.

INTRODUCTION

Since the introduction of the red swamp crayfish *Procambarus clarkii* in the early 1980s into the Egyptian fresh water systems for aquaculture from the United States of America *(1)*, it has been rapidly expanded in all aquatic ecosystems including streams, ponds and marshes with polluted or clean water. *P. clarkii* becomes successfully adapted to the new habitats and become an important component of the local aquatic fauna *(2, 3)*.

Procambarus clarkii stands as an important food in many parts of the world, being a rich source of protein *(4)*. In Egypt, it has been consumed in few areas, being cheaper than other decapods. Meanwhile, it causes a lot of damage to the fisheries of the Nile possibly eating the fry and the young fish and damaging the nets of fishermen *(4)*.

Heavy metals are common pollutants in large industrial cities *(5, 6)*. Despite the low concentrations of heavy metals in the surrounding medium, aquatic organisms take them up and accumulate in their soft tissues to concentrations several folds than those of ambient levels *(7)*.

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 and tend to accumulate metals in their tissues.*(8, 9)*.

Therefore, this study was planned to determine the concentration of some heavy metals (Zn, Pb, Cu, Ag, Ni and Hg) in crayfish (*Procambarus clarkii*) as a biological indicator of aquatic pollution.

MATERIAL AND METHODS

1-Crayfish samples

A total number of 120 crayfish (*Procambarus clarkii*) (Fig.1) were collected from natural water sources from 3 different localities, Zagazig, Sharkia Province, Benha, Qalyubia Province and Meetghamr, Dakahlia Province and immediately taken to the laboratory where kept frozen until the samples were prepared for digestion and analysis.

1.1-Digestion of samples

The frozen crayfish were defrosted and divided into two equal groups for each locality (A) and (B), each have 20 crayfish. Digestion of each group was proceeded according to the recommended method ***(10).*** Two grams from each crayfish sample were digested with 10 ml of analytical grade nitric/perchloric acid mixture (4:1) in clean acid washed digestion flask. Initial digestion was performed at room temperature for 3-4 hours, followed by careful heating in water bath at 40-45°C. for 1 hour to prevent frothing . The temperature was then raised to 70-80°C. with gentle shaking until the digestion was completed (within 3 hours). The resulting digests were allowed to cool at room temperature and diluted up to 20 ml with deionized water, then filtered through Whatman filtered paper No.1. Blank and standard solutions were also prepared and analyzed for quality control purpose.

2-Water samples

Two water samples were collected from each locality and at the same time where the crayfish samples were collected.

2.1-Collection of water samples

Two clean sample flasks of 1 liter capacity, equipped with glass stopper and rinsed several times with sampled water from each locality. Water samples were taken a hand breadth below the surface of water and the flasks were labeled with the sample number and immediately taken to the laboratory. The water samples were kept in refrigerator until they were prepared for analysis.

2.2-Preparation of water samples

The preparation of water samples for heavy metals analysis was conducted ***(11).*** The collected water samples were filtered through 0.45 micromembrane filter. One hundred ml of filtrate was measured and collected in clean glass bottles, preserved by 0.3 ml of nitric acid and kept till the metal analysis was performed.

3-Heavy metal analysis

Determination of heavy metal concentrations was conducted at the Central Laboratory, Faculty of Veterinary Medicine, Zagazig University, by using Buck Scientific Atomic Absorption Spectrophotometer 210 VGP, equipped with background corrector, autosampler and recorder. Copper, mercury, zinc, silver, lead and nickel were determined in crayfish and water samples using Airl Acetylene Flame (AAS).

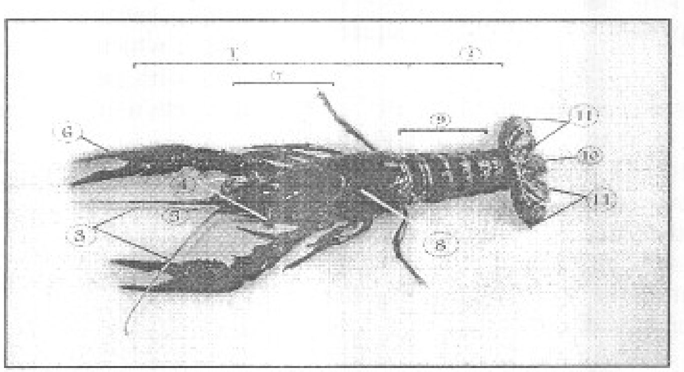

Fig. 1. Crayfish (*Procambarus clarkii*).
1. Cephalothorax, 2. Tail, 3. Antennae, 4. Eyes, 5. Rostrum, 6. Chelipeds, 7. Peripods, 8. Carapace, 9. Abdomen, 10.Uropod, 11. Telsons.

RESULTS AND DISCUSSION

1.Concentration of heavy metals in water and crayfish samples

The results demonstrated in Table 1 revealed that the mean values of Zn, Pb, Cu, Ag, Ni and Hg in Zagazig water samples were : 0.215±0.185, 0.15±0.0, 0.015±0.005, 0.01±0.01, 0.10±0.10 and 0.735±0.045 respectively; while in Benha were: 0.0004 ± 0.0001, 0.0061 ± 0.0002, 0.0035 ± 0.0005, 0.00005 ± 0.00005, 0.0055 ± 0.0005 and 0.58 ± 0.04 respectively; and in Meetghamr were: 0.0008 ± 0.0001, 0.0126 ± 0.0004, 0.004 ± 0.0005, 0.00015 ± 0.00005, 0.005 ± 0.001 and 0.29 ± 0.04 respectively. The results of water analysis revealed that the concentration of lead was significantly higher in Zagazig followed by Meetghamr and then Benha ($p \leq 0.05$). Also the concentration of mercury was significantly higher in Zagazig and Benha than Meetghamr ($p \leq 0.05$), while the other elements showed non significant differences between the examined localities.

It was found that the values of mercury and lead concentrations in water samples were nearly the same as those previously reported ***(12).*** Also the value of lead concentration was nearly the same as that cited ***(13),*** while there were differences between the concentration of other heavy metals in the current study in comparing with those recorded by several investigators ***(14-17)*** These may be attributed to the different concentrations of metals in water depending on the seasonal variations, the difference of the localities, and the types of discharges.

The concentration of these heavy metals in crayfish samples in Zagazig were 1.205 ± 0.095, 0.375 ± 0.075, 0.625 ± 0.125, 0.075 ± 0.025, 0.255 ± 0.245 and 1.385 ± 0.405 respectively; and in Benha they were 1.938 ± 0.491, 0.917 ± 0.045, 2.8 ± 0.725, 0.0625 ± 0.0125, 0.587 ± 0.0625 and 0.875 ± 0.055 respectively, while in Meetghamr they were 2.251 ± 0.196, 0.795 ± 0.228, 3.3125 ± 0.387, 0.0625 ± 0.0375, 0.437 ± 0.0375 and 0.74 ± 0.09 respectively. The results of the crayfish analysis revealed that the concentration of copper was significantly higher in crayfish obtained from Benha and Meetghamr than those obtained from Zagazig ($p \leq 0.05$), while the other elements showed non significant differences between the crayfish obtained from the examined localities.

The concentrations of copper and zinc in crayfish samples in the current study were nearly the same as those reported in a similar study ***(18).*** While there were differences between the concentration of other heavy metals in the current study in comparing with those cited by several investigations ***(16, 19, 20, 21).*** These differences may be attributed to the seasonal variations and the different localities from which it obtained, these localities differs from each others according to the types of discharges. Also it can be attributed to the different parts of crayfish that analyzed. Another reason of these differences was the fact that the crayfish itself make molting to its exoskeleton several times during its life, these exoskeletons that molted contained some of the accumulated heavy metals.

2-The obtained results in comparing with the permissible limits

Comparing the obtained results of water analysis with those of the permissible limits, it was found that : Pb, Ni and Hg in Zagazig were above the permissible limits; and Hg in Benha and Meetghamr was above the permissible limits. Regarding to the obtained results of crayfish analysis, it was found that : Ag and Hg in Zagazig were above the permissible limits; and Pb, Ag, Ni and Hg in Benha and Meetghamr were above the permissible limits (Table, 2).

The obtained results were nearly similar to that previously obtained ***(13, 16, 18).*** These differences comparing with results of others, may be due to heavily industrial discharges as well as sewage contamination and agricultural wastes. In addition, the crayfish has the ability to accumulate the heavy metals in different parts of its body.

3-Correlation coefficient of heavy metals in water and crayfish

It was observed that there was a positive correlation ($p \leq 0.05$) between different heavy metals in water samples and the same heavy metal residues in crayfish samples (table, 3).

This result agreed with that which reported that crayfish can be used for monitoring of heavy metal contamination in aquatic ecosystem due to their ability to accumulate and retain them rapidly in their tissues for a long period of time. Similar findings were reported by many authors ***(22-25).***

Table 1. Concentrations of heavy metals (ppm) in water and crayfish samples among the different localities (one-way ANOVA, p≤0.05).

Heavy Metals	Zagazig (Sharkia Province)							
	Water samples				Crayfish samples			
	Min.	Max.	Mean	S.E.±	Min.	Max.	Mean	S.E.±
Zn	0.03	0.4	0.215[a]	0.185	1.11	1.3	1.205[a]	0.095
Pb	0.15	0.15	0.15[a]	0.00	0.3	0.45	0.375[a]	0.075
Cu	0.01	0.02	0.015[a]	0.005	0.5	0.75	0.625[b]	0.125
Ag	0.00	0.02	0.01[a]	0.01	0.05	0.1	0.075[a]	0.025
Ni	0.00	0.2	0.10[a]	0.10	0.01	0.5	0.255[a]	0.245
Hg	0.69	0.78	0.735[a]	0.045	0.98	1.79	1.385[a]	0.405

Heavy Metals	Benha (Qalyubia Province)							
	Water samples				Crayfish samples			
	Min.	Max.	Mean	S.E.±	Min.	Max.	Mean	S.E.±
Zn	0.0003	0.0005	0.0004[a]	0.0001	1.447	2.43	1.938[a]	0.491
Pb	0.005	0.0063	0.0061[c]	0.0002	0.872	0.962	0.917[a]	0.045
Cu	0.003	0.004	0.0035[a]	0.0005	2.075	3.525	2.8[a]	0.725
Ag	0.00	0.0001	0.00005[a]	0.00005	0.05	0.075	0.0625[a]	0.0125
Ni	0.005	0.006	0.0055[a]	0.0005	0.525	0.65	0.587[a]	0.0625
Hg	0.54	0.62	0.58[a]	0.04	0.82	0.93	0.875[a]	0.055

Heavy Metals	Meetghamr (Dakahlia Province)							
	Water samples				Crayfish samples			
	Min.	Max.	Mean	S.E.±	Min.	Max.	Mean	S.E.±
Zn	0.0007	0.0009	0.0008[a]	0.0001	2.055	2.447	2.251[a]	0.196
Pb	0.012	0.013	0.0126[b]	0.0004	0.567	1.022	0.795[a]	0.228
Cu	0.004	0.005	0.004[a]	0.0005	2.925	3.7	3.3125[a]	0.387
Ag	0.0001	0.0002	0.00015[a]	0.00005	0.025	0.1	0.0625[a]	0.037
Ni	0.004	0.006	0.005[a]	0.001	0.4	0.475	0.437[a]	0.037
Hg	0.25	0.33	0.29[b]	0.04	0.65	0.83	0.74[a]	0.09

Means within the same column carrying different letters are significant at (p≤0.05).

Min. = Minimum.

Max. = Maximum.

S.E.± = ± Standard Error.

Table 2. Comparison between heavy metals in the examined water and crayfish samples from the examined localities, and the permissible limits (ppm).

Heavy Metals	Permissible limits		Zagazig		Benha		Meetghamr	
	Water samples	Crayfish samples	Water sample	Crayfish sample	Water sample	Crayfish sample	Water sample	Crayfish sample
Zn	5.00[1] 0.003[8] 0.005[9]	50[5],[6],[8]	0.215	1.205	0.0004	1.938	0.0008	2.251
Pb	0.050[1] 0.1[8] 0.02[9]	0.5[6],[8] 1.5-2[10]	0.15*	0.375	0.0061	0.917*	0.0126	0.795*
Cu	1.00[1],[8] 0.006[9]	20[6],[7],[8] 10-30[10]	0.015	0.625	0.0035	2.8	0.004	3.3125
Ag	0.1[2] 0.05[3]	0.003[8]	0.01	0.075*	0.00005	0.0625*	0.00015	0.0625*
Ni	0.02[4]	0.3[8]	0.10*	0.255	0.0055	0.587*	0.005	0.437*
Hg	0.001[1] 0.004[8] 0.0002[9]	0.5[6],[8],[10]	0.735*	1.385*	0.58*	0.875*	0.29*	0.74*

(1) WHO **(1984)** *(26)* (2) Canadian or BC Reg 230/92, & Sch 120 **(2001).** ***(27)***
(3) U.S. Environmental Protection Agency Drinking Water Standards **(2006).** ***(28)***
(4) WHO **(1998)** *(29)* (5) Food Stuff: Cosmetics and Disinfectants **(1972)** ***(30)***
(6) E.O.S.Q.C.**(1993)** ***(31)***
(7) Boletin Official del Estado, Spain (1991), in: Daoud et al., **(1999)** ***(32)***
(8) Turkey Fishery Regulations **(1995).** ***(33)***
(9) Australia Water Quality Standards **(2005).*(34)*** (10) FAO **(1983)**.*(35)*
* Over the permissible limits.

Table 3. Correlation coefficient of heavy metals in water samples with the same heavy metal residues in crayfish samples.

Heavy Metals in crayfish \ Heavy Metals in water	Zn	Pb	Cu	Ag	Ni	Hg
Zn	0.462					
Pb		0.840*				
Cu			1.000			
Ag				0.544		
Ni					0.190	
Hg						0.762

* Correlation is significant at the 0.05 level (2-tailed).

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الملخص العربى

تركيزات بعض المعادن الثقيلة في استاكوزا المياه العذبة (أسماك الكراى) كمؤشر حيوي لتلوث المياه

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في هذه الدراسة تم استخدام عدد ١٢٠ عينة من استاكوزا المياه العذبة و ٦ عينات مياه تم تجميعهم من ثلاث أماكن مختلفة و هي : الزقازيق (محافظة الشرقية) ، بنها (محافظة القليوبية) و ميت غمر (محافظة الدقهلية) و ذلك لتحديد نسب الزنك ، الرصاص ، النحاس ، الفضة ، النيكل و الزئبق باستخدام جهاز طيف الامتصاص الذرى.

أوضحت نتائج الدراسة ما يلي :-

١- أن عينات المياه المختبرة من الزقازيق أظهرت زيادة ملحوظة في نسبة الرصاص يليها ميت غمر ثم بنها و أيضا أظهرت نسبة الزئبق زيادة ملحوظة في الزقازيق و بنها عن ميت غمر. بينما باقي العناصر في المناطق المختبرة لم تظهر أي زيادة ملحوظة.

٢- أن عينات استاكوزا المياه العذبة المختبرة من بنها و ميت غمر أظهرت زيادة ملحوظة في نسبة النحاس عن العينات المختبرة من الزقازيق. بينما باقي العناصر في المناطق المختبرة لم تظهر أي زيادة ملحوظة.

٣- وجد أن هناك بعض العناصر في عينات المياه و استاكوزا المياه العذبة المختبرة في المناطق المختلفة تزيد عن الحد المسموح به.

٤- وجد أن هناك ارتباط وثيق بين العناصر المختبرة في المياه و مثيلاتها في استاكوزا المياه العذبة في المناطق المختلفة.