Determination of Some Heavy Metals in Shellfish

Saadia, M. H. Mohammed., Abo Samaha, O. R., Abdel-Nabey, A. A. & Khalil, M. K. M.

Food Science and Technology Dept., Fac. of Agric., El-Shatby, 21545,

Alexandria Univ., Alexandria, Egypt

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ABSTRACT

The concentrations of Cu, Cd, Pb, As and Hg in the edible portions of four species of Egyptian common invertebrates namely, undulate venus (*Paphia undulata*), peregrine shrimp (*Metapenaeus stebbingi*), Pelagic swim crab (*Portunus pelagicus*) which were collected from two different fishing areas (El- Max and Abu-Qir Bay) and wedge clam (*Donax trunculus*) which was collected from Edkou beach, were determined by atomic absorption spectrophotometer technique. Analysis of variance for heavy metal levels as affected by location, season and species and their first order interaction (location × season, location × species and seasons × species of shellfish) indicated that, the concentrations of Pb, As and Hg showed significant differences, while Cu and Cd concentrations showed no significant differences between locations (El-Max and Abu-Qir Bay). All heavy metal concentrations showed significant differences between seasons, except Cu concentrations which showed no significant differences. All heavy metal concentrations showed significant differences between the studied species. The ANOVA data showed no significant interactions between (location × season), (location × shellfish species) and (seasons × shellfish species) in the concentrations of heavy metals, except As content in interaction (seasons × shellfish species). The highest concentrations of Cu, Cd, Pb, As and Hg were recorded in the summer season, while the lowest levels were observed during the spring season. The highest concentrations of Cu, Cd, Pb, As and Hg were found in the edible portions of the three shellfish samples obtained from Abu-Qir Bay. No significant differences with respect to the levels of Cu and Cd between the two sampling areas were detected. The highest concentrations of Cd, Pb, As and Hg were obtained in the edible portions of *Paphia undulata*, while *Portunus pelagicus* contained the highest level of Cu. On the other hand, the lowest concentrations of Cd, Pb, and As were recorded for the edible portions of *Metapenaeus stebbingi*, while *Paphia undulate* had the lowest concentration of Cu and *Portunus pelagicus* had the lowest concentrations of Hg.

Key words: Heavy metals, shellfish species, locations, seasonal variations.

INTRODUCTION

Environmental pollution represents a major problem in both developed and underdeveloped countries. Egypt is one of the countries which suffer from high biosphere pollution (air, soil and water). Many ecological changes occur in water as a result of human activities, including agricultural, industrial and municipal wastes (Atta *et al*., 1997).

Seas and Oceans, which cover 70% of the world's surface, are one of the man's great hopes for future food supplies. As human populations multiply and industrialization increases, the problems of environmental pollution become more critical. Heavy metals enter the aquatic environment naturally through weathering of the earths crust. In addition to geological weathering, human activities have also introduced large quantities of metals to local water bodies, thereby disturbing the natural balance in the ecosystem (Forstner & Wittmann, 1983).

Sea foods contain essential amino acids, fatty acids, protein, vitamins and minerals. Among sea foods, fish are commonly consumed and, hence, are a connecting link for the transfer of toxic heavy metals in human being. Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards (Puel *et al*., 1987).

Fish is the major part of the human diet and it is therefore not surprising that numerous studies

have been carried out on metal pollution in different species of edible fish. Industrial effluents, agricultural runoffs, transport, burning of fossil fuels, animal and human excretions and geologic weathering and domestic waste contribute to the heavy metals in the water bodies. Heavy metals are critical in this regard because of their easy uptake into the food chain and bioaccumulation processes (Raja *et al*., 2009).

Heavy metals acquired through the food chain as a result of pollution are potential chemical hazards, threatening consumers. At low levels, some heavy metals such as copper, zinc, iron and manganese are essential for enzymatic activity and many biological processes. Other metals, such as cadmium, mercury, arsenic and lead have no known essential role in living organisms, and are toxic at even low concentrations. The essential metals also become toxic at high concentrations (Al-Weher, 2008).

Consumption of fish is very popular among people all around the world because it has high protein content, low saturated fatty acids, and high omega fatty acids content. Processing steps may change the concentration of heavy metals in fish before consumption (Ganjavi *et al*., 2010).

The variations of heavy metal concentrations in fish from different areas of the world may be possibly due to differences in metal concentrations, chemical characteristics of water from which fish were sampled, ecological needs, metabolism and feeding patterns of fishes and also the season in which studies were carried out (Bahnasawy *et al*., 2009). Different location and season have an effect on heavy metals content in fish samples (Gomaa, 1995).

In Egypt, fish, shellfish and fish products are considered to be one of the popular foods. Alexandria is one of the major industrial centers, and its coast is considered to be one of the main summer resort and fishing area. The Mediterranean is subject to heavy discharges of pollutants from numerous industrial processes. Among these industries are leather tanning, metallic transformation, oil refineries and petroleum terminals and organic and/or inorganic chemical industries which might affect the concentration of metals in salt water fish. Many factories occur in and around Alexandria such as Egyptian Liquefied Natural Gas (ELNG), Rashpetco, Gasco, Wepco, Petroget (shipyard), Abu-Qir fertilizers, Alexandria fertilizers, National Paper and Racta company. Most of the factories wastes are discarded into the Mediterranean Sea.

To the best of our knowledge, little attention has been paid to study heavy metals pollution in some shellfish species caught from Al-Max area, Abu-Qir Bay and Edkou beach which represent the main fishing area at and around Alexandria coast. So, the present study was planned to throw a light on the determination of some heavy metals in shellfish.

MATERIALS AND METHODS

Materials

Fresh samples of peregrine shrimp (*Metapenaeus stebbingi*), Pelagic swimcrab or red crab (*Portunus pelagicus*), undulate venus (*Paphia undulata*) were collected from the fishing areas in El-Max and Abu-Qir Bay. Wedge clam or truncate donax (*Donax trunculus*) was collected from Edkou beach.

These samples have been collected within the four different seasons during 2009 (February to November). Shellfish species were immediately put up into insulated boxes containing crushed ice and transferred to the laboratory for chemical analysis. The edible portion of the meat from each sample was removed, homogenized using a blender (Braun type: 4262), stored frozen at -18^{o}C in polyethylene bags until analyzed.

Methods

Moisture content was determined by drying about 5g sample at103±2 ^{o}C to constant weight as described by Less (1975).

The heavy metals (copper "Cu", cadmim "Ca", lead "Pb", arsenic "As" and mercury "Hg") concentration in the samples were determined after digestion using Atomic Absorption Spectroscopy (AAS) according to the method dsecribed by Vitosevic *et al* (2007) as follows: A sample of 1-3 g was put into a 100 ml Kjeldahl flask with 10 ml of conc. sulphuric acid (H_2SO_4) and nitric acid (HNO_3) (1:3). After thermolysis and heating until discoloration, the solution was cooled, filtered using ashless filter paper (Whatman 43) then diluted to 50 ml with deionized water. This solution was analyzed using AAS (SHIMADZU Atomic Absorption Spectrophotometer AA-6800 where the flame unit was used together with the auto sampler SHIMADZU ASC-6100).

Statistical analysis

Three factors experiment was concluded using

one replicate design in completely randomized design. The three-factors interaction was used as an error as advised by Petersen (1985). The analysis of variance and the mean comparison were computed using SAS program (for fresh shellfish samples).

RESULTS AND DISCUSSION

Concentrations of heavy metals in the edible portions of shellfish samples

Copper content

The results in Table (1) indicate that Cu concentrations in the edible portions of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus* collected from El-Max varied from 39.55 to 67.83, 56.28 to 82.99 and 80.81 to 130.59, whereas it ranged between 41.27 to 68.95, 63.10 to 83.57 and 106.72 to 137.69 mg/kg, (dry wt.) in the samples collected from Abu-Qir Bay.

The edible portions of *Paphia undulata* collected from Abu-Qir Bay during the summer season contained the highest concentrations of Cu, while the lowest concentrations of Cu were recorded during the spring season (Table 1).

The highest values of Cu were detected in the edible portions of *Metapenaeus stebbingi* collected from El-Max and Abu-Qir Bay during summer season. However the minimum values of Cu were recorded during spring and autumn.

The edible portions of *Paphia undulata* collected from El- Max area (Table 1) recorded the lowest concentration of Cu during the spring season. No significant differences in Cu content were recorded with respect to the edible portions of *Paphia undulata* and *Metapenaeus stebbingi* obtained from El-Max and Abu-Qir Bay during the different seasons.

On the other hand, the maximum concentrations of Cu were recorded for the edible portions of *Paphia undulata* obtained from El-Max area during summer and winter seasons with no significant differences with those obtained from Abu-Qir Bay during summer and autumn seasons.

The edible portions of *Portunus pelagicus* collected from Abu-Qir Bay during the autumn season contained high concentrations of Cu, while the lowest concentrations of Cu were recorded during the spring season from El-Max area.

In accordance with the results obtained here, Hiraoka (1991) found that the Cu content in Hiroshima Bay oyster was 140 µg/g, (dry wt.). On the other hand, Cu content in the edible portions of *Phaphia undulata* was lower than those reported by Brown & McPherson (1992) in Sydney rock oyster from the Georges River, Australia. Further, Aboul Dahab (1991) showed that the Cu levels in Qatar shellfish *Portunus pelagicus* and *Penaeus semisulcatus* were as 1920 and 2474 µg /kg, (fresh wt.), respectively. The results obtained in the present study are not in agreement with those obtained by Lin & Hsieh (1999), Mansour & Sidky (2002), Usero *et al*. (2005), Das *et al.* (2007), Soegianto & Hamami (2007) and Gokoglu *et al.* (2008).

All concentrations of Cu (wet wt.) found in the edible portion of *Paphia undulata*, *Metapenaeus*

Table 1: Copper content (mg/kg) in the edible portions of shellfish samples

Sampling area	Season	*Paphia undulata*			*Metapenaeus stebbingi*			*Portunus pelagicus*		
		Moisture (%)	Wwb*	Dwb**	Moisture (%)	Wwb*	Dwb**	Moisture (%)	Wwb*	Dwb**
El-Max	winter	83.88±0.66	10.39	64.47^{a}	81.41±0.50	13.64	73.38^{a}	81.93±0.66	15.36	85.04^{c}
	spring	85.04±0.13	5.92	39.55^{a}	76.05±0.24	13.48	56.28^{a}	79.15±0.11	16.85	80.81^{c}
	summer	87.45±0.21	8.52	67.83^{a}	79.62±0.25	16.92	82.99^{a}	80.88±1.21	24.97	130.59^{ab}
	autumn	82.22±0.28	9.02	50.73^{a}	78.64±0.41	12.37	57.91^{a}	75.60±0.45	31.40	128.71^{ab}
Abu-Qir Bay	winter	82.10±0.20	7.42	41.42^{a}	78.49±0.23	16.08	74.64^{a}	74.36±0.19	33.86	132.07^{ab}
	spring	84.92±0.16	6.22	41.27^{a}	76.50±0.17	19.51	83.01^{a}	82.36±0.23	19.37	109.82^{abc}
	summer	87.50±0.38	8.62	68.95^{a}	79.65±0.39	17.01	83.57^{a}	77.10±0.68	24.44	106.72^{bc}
	autumn	83.91±0.07	8.40	52.20^{a}	78.81±0.22	13.37	63.10^{a}	81.62±0.25	25.31	137.69^{a}
L. S. D	29.42									

*Wet weight basis **Dry weight basis

Means in the same column sharing the same letters are not significantly different, at P<0.05.

stebbingi and *Portunus pelagicus* collected from El-Max and Abu-Qir Bay (except *Portunus pelagicus* collected from Abu-Qir during winter season and from El-Max during autumn season) were below the maximum permissiable levels (30 mg/kg on wet weight basis) as recommended by FAO (1992).

Cadmium content

The results in Table (2) indicate that the total cadmium content (dry wt.) in the edible portions of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus* obtained from El-Max area varied from 3.13 to 6.38, 2.86 to 4.52 and 2.73 to 5.33 mg/kg, respectively.

On the other hand, it varied from 3.92 to 8.30 mg/kg in *Paphia undulata*, from 3.36 to 4.66 mg/kg in *Metapenaeus stebbingi* and from 4.34 to 5.81 mg/kg in *Portunus pelagicus* obtained from Abu-Qir Bay, respectively. The results indicated that the total Cd content in the edible portions of the three different shellfish samples obtained during the summer seasons from the two sampling areas were found to be the highest. On the other hand, the samples obtained during the spring season showed the lowest values. All levels of Cd of the edible Portions of *Metapenaeus stebbingi* obtained from the two sampling areas showed no significant differences. The same trend was noticed between the Cd levels of *Portunus Pelagicus* obtained from Abu-Qir Bay during different seasons as well as those obtained from El-Max area during summer and autumn seasons.

The results of total Cd content in different shellfish species obtained in the present study are higher than those reported by Aboul Dahab (1991), Hiraoka. (1991), Elston *et al.* (2005), Usero *et al*. (2005), Soliman (2006), Das *et al*. (2007), Soegianto & Hamami (2007), and Gokoglu *et al.* (2008), which may be attributed to the studied species and the location in which these species were collected. On the other hand, the results of Cd content in shrimp samples obtained in the present study are in a good agreement with those reported by Soliman (2006).

All concentrations of Cd found in the edible portion of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus* collected from El-Max and Abu-Qir Bay were below the maximum permissiable limits (3 mg/kg in crustacean seafood and 4 mg/kg for clam oyster and mussels molluscan shellfish) proposed by FDA (2001) and were below the maximum permissiable limits (2ppm) proposed by FAO (1992).

Notwithstanding, shellfish samples had higher concentrations of Cd than the permissiable limits (0.1 mg/kg) proposed by Egyption Organization for Standardization and Quality Control (EOSQC, 1993).

Lead content

The results in Table (3) indicate that the total concentrations of Pb in the edible portions of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus* obtained from El-Max area (mg/kg dry wt.) ranged between 186.15 to 302.21, 94.82 to 175.94 and from 111.51 to 146.81, respectively. On the other hand, the total levels of Pb varied from 188.36 to 412.46, 115.63 to 172.70 and from 119.97 to 220.19 (mg/kg dry wt.) in sample obtained from

Table 2: Cadmium content (mg/kg) in the edible portions of shellfish samples+

Sampling area	Season	*Paphia undulata*		*Metapenaeus stebbingi*		*Portunus pelagicus*	
		Wwb*	Dwb**	Wwb*	Dwb**	Wwb*	Dwb**
El-Max	winter	0.94	5.85^{bc}	0.58	3.10^{a}	0.55	3.05^{b}
	spring	0.47	3.13^{d}	0.69	2.86^{a}	0.57	2.73^{b}
	summer	0.80	6.38^{b}	0.92	4.52^{a}	1.02	5.33^{a}
	autumn	0.62	3.46^{d}	0.67	3.15^{a}	1.28	5.24^{a}
Abu-Qir Bay	winter	0.70	3.92^{d}	0.73	3.36^{a}	1.17	4.57^{ab}
	spring	0.66	4.37^{cd}	0.81	3.44^{a}	0.77	4.34^{ab}
	summer	1.04	8.30^{a}	0.95	4.66^{a}	1.33	5.81^{a}
	autumn	0.96	5.95^{bc}	0.73	3.44^{a}	1.04	5.67^{a}
L. S. D				1.85			

*Wet weight basis **Dry weight basis

Means in the same column sharing the same letters are not significantly different, at $P<0.05$.

+Moisture contents in shellfish samples were presented in Table (1)

Table 3: Lead content (mg/kg) in the edible portions of shellfish samples+

Sampling area	Season	*Paphia undulata*		*Metapenaeus stebbingi*		*Portunus pelagicus*	
		Wwb*	Dwb**	Wwb*	Dwb**	Wwb*	Dwb**
El-Max	winter	32.20	199.77^{c}	29.62	159.33^{ab}	20.15	111.51^{c}
	spring	27.84	186.15^{c}	22.71	94.82^{c}	24.88	119.29^{c}
	summer	37.94	302.21^{b}	35.86	175.94^{a}	28.07	146.81^{bc}
	autumn	34.38	193.30^{c}	26.89	125.91^{abc}	32.50	133.22^{c}
Abu-Qir Bay	winter	56.50	315.57^{b}	35.69	165.58^{ab}	30.76	119.97^{c}
	spring	28.41	188.36^{c}	27.17	115.63^{bc}	28.13	159.45^{bc}
	summer	51.56	412.46^{a}	35.15	172.70^{a}	50.43	220.19^{a}
	autumn	38.45	239.05^{c}	32.69	154.27^{ab}	36.03	196.00^{ab}
L. S. D				56.59			

*Wet weight basis **Dry weight basis

Means in the same column sharing the same letters are not significantly different, at P<0.05.

+Moisture contents in shellfish samples were presented in Table (1)

Abu-Qir Bay, respectively. The highest concentrations of Pb were recorded during the summer season, while the lowest values were noticed during the spring season in most cases.

Generally, all total concentrations of Pb in the studied samples obtained from the different locations were higher than those reported by Aboul Dahab (1991), Hiroaka (1991), Brown & McPherson (1992), Elston et al. (2005), Usero *et al.* (2005) and Das *et al*. (2007), either on dry or wet wt.basis.

The samples collected from El-Max and Abu-Qir Bay had higher concentrations of Pb (wet wt.) than the permissiable limits (2 mg/kg on wet weight basis) proposed by FAO (1992) and EOSQC (1993), (0.1 mg/kg, wet wt).

Arsenic content

The data in Table (4) indicate that the total concentrations of As in the muscles of *Paphia undulata* obtained from EL-Max area and Abu-Qir Bay varied from 9.68 to 13.55 and from 11.16 to 21.49 mg/kg (dry wt.), respectively. On the other hand, the total concentrations of As in muscles of *Metapenaeus stebbingi* obtained from the two sampling areas varied from 1.63 to 2.45 and from 2.00 to 2.71 (mg/kg dry wt.), respectively. Generally no significant differences were noticed among all the total concentrations of As obtained for *Metapenaeus stebbingi* from the two sampling areas.

Further, the total concentrations of As in the edible portions of *Portunus pelagicus* collected from El-Max area and Abu-Qir Bay during the dif-

Table 4: Arsenic content (mg/kg) in the edible portions of shellfish samples+

Sampling area	Season	*Paphia undulata*		*Metapenaeus stebbingi*		*Portunus pelagicus*	
		Wwb*	Dwb**	Wwb*	Dwb**	Wwb*	Dwb**
El-Max	winter	2.08	12.90^{b}	0.30	1.63^{a}	1.96	10.84^{d}
	spring	1.81	12.12^{bc}	0.43	1.79^{a}	1.18	5.66^{e}
	summer	1.70	13.55^{b}	0.50	2.45^{a}	2.73	14.28^{bc}
	autumn	1.72	9.68^{c}	0.35	1.64^{a}	3.18	13.05^{cd}
Abu-Qir Bay	winter	2.11	12.17^{bc}	0.43	2.00^{a}	3.36	13.09^{cd}
	spring	1.99	13.20^{b}	0.46	2.10^{a}	1.16	6.57^{e}
	summer	2.69	21.49^{a}	0.55	2.71^{a}	4.22	18.44^{a}
	autumn	1.80	11.16^{bc}	0.46	2.16^{a}	3.15	17.14^{ab}
L. S. D				3.01			

*Wet weight basis **Dry weight basis

Means in the same column sharing the same letters are not significantly different, at P<0.05.

+Moisture contents in shellfish samples were presented in Table (1)

ferent seasons ranged from 5.66 to 14.28 and from 6.57to 18.44 mg/kg (dry wt.), respectively. Meanwhile, the three studied shellfish samples obtained from the two different sampling areas during the summer season contained the highest concentration of As.

According to the results obtained by Elston *et al*. (2005), Usero *et al*. (2005), Das *et al*. (2007) and Soegianto & Homami (2007), the concentrations of As in different shellfish species obtained from different locations were lower than those found in the present study. On the other hand, the results of As content in *Paphia undulata* obtained in the present study agreed well with those reported by Hiraoka (1991).

All concentrations of As found in the edible portion of *Metapenaeus stebbingi* which collected from El-Max and Abu-Qir Bay and *Paphia undulata* (except summer samples from Abu-Qir Bay) were below the maximum permissiable levels (2 mg/kg, wet wt.) proposed by WHO (1996).

On the other hand, *Portunus pelagicus* had higher concentrations of As than the permissiable limits, except the samples collected from El-Max, during winter and spring and the samples collected during spring from Abu-Qir Bay which were below the permissiable limits.

Mercury content

The results in Table (5) reveal that the total mercury concentrations in the edible portions of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus* obtained from EL-Max area varied between 0.24 to 0.66, 0.14 to 0.33, 0.09 to 0.30, whereas the values of the samples obtained from Abu-Qir Bay were 0.29 to 0.67, 0.19 to 0.40 and 0.18 to 0.43 (mg/kg dry wt.), respectively.

Except the level of Hg of *Metapenaeus stebbingi,* obtained from Abu-Qir Bay during the winter season, the highest concentrations of Hg were found in the muscle of the three samples obtained from the two different sampling areas during the summer seasons.

The data in Table (5) also indicate that no significant differences were noticed among the total level of Hg obtained during the summer season from El-Max area and Abu-Qir Bay. On the other hand, no significant differences were noticed among the total concentration of Hg obtained during winter and spring seasons from the two different sampling areas for the studied samples except *Metapenaeus stebbingi*.

In accordance with the results obtained here, Telb (1981) found that the total concentrations of Hg in blue crab, deep-water pink shrimp and grooved carpet shell collected from El-Max varied between 0.147 to 0.35, 0.381 to 0.356 and 0.528 to 0.838 mg/kg, (wet wt.), respectively. While samples collected from Abu-Qir Bay varied from 0.077 to 0.29, 0.037 to 0.0846 and 0.05 to 0.068 mg/kg, (wet wt.), respectively.

The concentrations of Hg content in different locations as reported by Elston *et al*. (2005), Usero *et al*. (2005) and El-Moselhy (2006) in *Paphia undulata* and *Metapenaeus stebbingi* were lower than those obtained in the present study, while the Hg content in different shellfish species as reported by Soliman

Table 5: Mercury content (mg/kg) in the edible portions of shellfish samples+

Sampling area	Season	*Paphia undulata*		*Metapenaeus stebbingi*		*Portunus pelagicus*	
		Wwb*	Dwb**	Wwb*	Dwb**	Wwb*	Dwb**
El-Max	winter	0.05	0.33^{b}	0.03	0.14^{d}	0.02	0.13^{c}
	spring	0.04	0.27^{b}	0.04	0.16^{d}	0.02	0.09^{c}
	summer	0.08	0.66^{a}	0.07	0.33^{abc}	0.06	0.30^{ab}
	autumn	0.04	0.24^{b}	0.04	0.21^{bcd}	0.05	0.21^{bc}
Abu-Qir Bay	winter	0.05	0.29^{b}	0.09	0.40^{a}	0.05	0.18^{bc}
	spring	0.06	0.37^{b}	0.08	0.35^{ab}	0.04	0.22^{bc}
	summer	0.08	0.67^{a}	0.07	0.33^{abc}	0.10	0.43^{a}
	autumn	0.06	0.35^{b}	0.04	0.19^{cd}	0.05	0.30^{ab}
L. S. D	0.15						

*Wet weight basis **Dry weight basis

Means in the same column sharing the same letters are not significantly different, at $P<0.05$.

+Moisture contents in shellfish samples were presented in Table (1)

(2006) and Das *et al*. (2007) in crab were higher than those obtained in the present study. These variations in Hg levels may be mainly due to growing location, season, size, sex and species variations.

On the other hand, the results of Hg content in the present study were in agreement with those reported by El-Moselhy (2006) and Soliman (2006) in *Portunus pelagicus* and *Metapenaeus stebbingi* samples, respectively.

Generally, all the total concentrations of Hg found in edible portions of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus* collected from El-Max and Abu-Qir Bay were below the maximum permissiable limit (0.5 mg/kg) regulated by EOSQC (1993), while FDA (2001) has set a maximum total mercury level of 1mg/kg.

As a conclusion, the overall mean values of the studied heavy metals of the edible portions of the three different species collected from the two different sampling areas can be arranged in a descending order as follows: Pb > Cu > As > Cd > Hg.

Concentrations of heavy metals (mg/kg) in the edible portions of *Donax trunculus*

The concentrations of heavy metals (mg/kg dry wt.) in the edible portions of *Donax trunculus* obtained from Edkou beach are shown in Table (6).

The concentrations of Cu, Cd, Pb, As, Hg during the different seasons (mg/kg dry wt.) varied between 25.72 to 144.65, 2.53 to 7.43, 174.67 to 496.09, 6.73 to 14.29 and from 0.24 to 0.90 (on dry wt. basis), respectively.

The results indicated that the maximum concentrations of Cu were recorded in the edible portions of *Donax trunculus* obtained during the winter season. On the other hand, the highest concentrations of Cd, Pb and Hg were observed during the spring season. Furthermore, the highest concentrations of As were recorded during the autumn season.

Telb (1981) found that the concentration of Hg in wedge clam (*Donax trunculus*) ranged between 0.072 to 1.054 mg/kg (wet weight). These values are higher than the Hg concentrations found in the present study.

The results obtained in the present study are not in agreement with those reported by Beldi *et al*. (2006). They found that the highest values of Cd, Pb and Cu were recorded in the summer season. On the other hand, the levels of Cu, Cd, Pb and As reported by Elston *et al.* (2005), Usero *et al*. (2005) are lower than those obtained in the present study.

All concentrations of Cu found in the edible portion of Donax trunculus (except winter sample) were below the maximum permissiable limit proposed by FAO (1992). Whereas the levels of Cd were below the maximum permissiable limit proposed by FAO (1992) and FDA (2001). Donax trunculus had higher levels of Pb than the permissiable limits (2 mg/kg) proposed by FAO (1992). Generally, all the total levels of Hg were below the maximum permissiable limit (0.5 mg/kg) regulated by EOSQC (1993). FDA (2001) has set a maximum total mercury concentration of 1ppm. Winter and autumn samples had higher concentrations of As, while spring and summer samples had lower concentrations of As than permissiable limits (2 mg/kg) proposed by WHO (1996).

Table 6: Concentrations of heavy metals (mg/kg) in the edible portions of *Donax trunculus*

Season	Moisture (%)	Cu	Cd	Pb	As	Hg
Winter	76.03±0.30	34.68*	0.61*	41.87*	2.77*	0.08*
		144.65**	2.53**	174.67**	11.57**	0.31**
Spring	78.13±0.17	17.32*	1.62*	108.49*	1.47*	0.20*
		79.20**	7.43**	496.09**	6.73**	0.90**
Summer	82.98±0.24	4.38*	0.73*	57.12*	1.53*	0.04*
		25.72**	4.31**	335.53**	8.98**	0.24**
autumn	79.18±0.70	10.43*	1.26*	44.37*	2.97*	0.06*
		50.07**	6.04**	213.11**	14.29**	0.28**

*Wet weight basis **Dry weight basis.

Statistical analysis of some factors affecting the concentrations of heavy metals.

Analysis of variance for heavy metal levels as affected by location, season and shellfish species samples and their first order interaction (location × season, location × species and seasons × species of shellfish) are tabulated in Table (7).

It is obviously that, the concentrations of Pb, As and Hg showed significant differences, while Cu and Cd levels showed no significant differences between locations (El-Max and Abu-Qir Bay). All heavy metal concentrations showed significant differences between seasons, except Cu concentration which showed no significant differences. All heavy metal concentrations showed significant differences between the studied species.

The ANOVA data explored no significant interaction (location × season, location × shellfish species and seasons × shellfish species) in the concentrations of heavy metal levels, except As content in interaction seasons × shellfish species.

Seasonal variation of heavy metals recorded in the shellfish samples

Table (8) shows that the maximum concentrations of Cu, Cd, Pb, As and Hg the levels of 90.11, 5.83, 238.39, 12.15 and 0.45 mg/kg (dry wt.), respectively were recorded in the summer season. These results were in agreement with those obtained by Hoza (1991) and Gomaa (1995) with respect to the levels of Cd and also with those obtained by Swaileh & Adelung (1995), Attwa (1997), El-Moselhy & Yassien (2005), Beldi *et al.*(2006) and Bahnasawy *et al.*(2009) with respect to the concentrations of Cu, Cd and Pb.

Table (8) also shows that the maximum concentration of Cu was recorded in the summer season with no significant differences from those obtained during winter and autumn seasons.

The increase in the concentration of metals during the summer corresponds to the main growth period of these species where growth is most quickly, in length (June – September) and stops grow-

Table 7: F-values for analysis of variance of heavy metals as affected by location, season and shellfish species+ and their first order interaction

Factor	Cu		Cd		Pb		As		Hg	
	df	F value	df	F value	df	F value	df	F value	df	F value
Location (L)	1	$1.11^{n.s}$	1	$3.96^{n.s}$	1	13.55**	1	9.37*	1	7.85*
Season (S)	3	$2.22^{n.s}$	3	7.17*	3	11.72**	3	12.51**	3	11.61**
Shellfish species (V)	2	35.60**	2	6.14*	2	37.74**	2	137.17**	2	11.10**
L*S	3	$0.83^{n.s}$	3	$0.52^{n.s}$	3	$0.49^{n.s}$	3	$1.74^{n.s}$	3	$0.49^{n.s}$
L*V	2	$0.95^{n.s}$	2	$0.34^{n.s}$	2	$1.94^{n.s}$	2	$1.57^{n.s}$	2	$0.44^{n.s}$
S*V	6	$1.10^{n.s}$	6	$1.01^{n.s}$	6	$3.27^{n.s}$	6	7.00*	6	$2.16^{n.s}$

+Shellfish species (*Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus*)

*Significant at P< 0.05 ** Significant at P< 0.01 n.s: P> 0.05.

Table 8: Concentrations of heavy metals (mg/kg dry wt.) of shellfish species+ obtained during, 2009

Season	Cu	Cd	Pb	As	Hg
Winter	78.50^{ab}	3.97^{b}	178.62^{b}	8.76^{bc}	0.25^{b}
Spring	68.46^{b}	3.48^{b}	143.93^{b}	6.91^{c}	0.24^{b}
Summer	90.11^{a}	5.83^{a}	238.39^{a}	12.15^{a}	0.45^{a}
Autumn	81.72^{ab}	4.49^{b}	173.62^{b}	9.14^{b}	0.25^{b}
L. S. D	20.81	1.31	40.01	2.13	0.11

Means between columns sharing the same letters are not significantly, at P<0.05.

+Shellfish species (*Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus*).

ing in the late of autumn when maturity is reached (Swalieh & Adelung, 1995). On the other hand, Bahnasawy *et al.* (2009) reported that variation of the metal concentration at a given site may be attributed to seasonal changes of the organisms, tissue weights rather than to any variability in absolute metal content of the organism. Mansour & Sidky (2002) found that the maximum level of Cu was recorded in the autumn season.

The results in Table (8) indicate that the minimum concentrations of Cu, Cd, Pb, As and Hg (68.46, 3.48, 143.93, 6.91 and 0.24) mg/kg (dry wt.), respectively were observed during the spring season. These results are in agreement with those obtained by Gomaa (1995) and Mansour & Sidky (2002) with respect to the levels of Pb. The lowest concentrations of Cd were found during the spring season with no significant differences in comparison with those obtained during winter and autumn seasons (Table 8).

No significant differences were observed between the levels of Cu, Cd, Pb and Hg for the edible portions of *Paphia undulata*, *Metapenaeus stebbingi* and *Portunu pelagicus* obtained during winter, spring and autumn seasons from the two different sampling areas (El-Max and Abu-Qir Bay).

Hoza (1991) showed that the minimum concentrations of Cd were recorded during the winter season. Further, Gomaa (1995) reported that the minimum levels of Cd were recorded in the autumn season. Also, they reported that the lowest levels of Cu were obtained during the summer season.

Mansour & Sidky (2002) reported that the seasonal variation of heavy metals (as total values), revealed the following descending order: Summer > autumn > winter > spring.

Effect of sampling area (location) on concentrations of heavy metals of the shellfish samples

Table (9) shows that the maximum concentrations of Cu, Cd, Pb, As and Hg of the three studied samples obtained from Abu-Qir Bay were 82.87, 4.82, 204.92, 10.18 and 0.34 mg/kg (dry wt.), respectively. No significant differences were noticed with respect to the concentrations of Cu, Cd between the two sampling area.

Telb (1981) found that Hg concentrations of blue crab, deep-water shrimp and grooved carpet shell were higher in El-Max than in Abu-Qir Bay. On the other hand, El-Moselhy (2006) stated that Hg concentrations in El-Max Bay were higher than Abu-Qir Bay.

Effect of shellfish species on concentrations of heavy metals

Table (10) shows that the maximum concentrations of Cd, Pb, As and Hg were found in the edible portions of *Paphia undulata*, whereas *Portunus pelagicus* contained the maximum concentrations of Cu. On the other hand, the lowest levels of Cd, Pb, and As were recorded in the edible portions

Table 9: Concentrations of heavy metals (mg/kg dry wt.) of shellfish species+ obtained during 2009

Location	Cu	Cd	Pb	As	Hg
El-Max	76.52[a]	4.07[a]	162.36[b]	8.30[b]	0.26[b]
Abu-Qir Bay	82.87[a]	4.82[a]	204.92[a]	10.18[a]	0.34[a]
L. S. D	14.71	0.93	28.29	1.50	0.07

Means between columns sharing the same letters are not significantly, at at P<0.05.

+Shellfish species (*Paphia undulata*, *Metapenaeus stebbingi* and *Portunus pelagicus*).

Table 10: Concentrations of heavy metals (mg/kg dry wt.) of shellfish species, during 2009

Shellfish samples	Cu	Cd	Pb	As	Hg
Paphia undulate	53.30[c]	5.17[a]	254.61[a]	13.28[a]	0.40[a]
Metapenaeus stebbingi	71.86[b]	3.57[b]	145.52[b]	2.06[b]	0.26[b]
Portunus pelagicus	113.93[a]	4.59[ab]	150.79[b]	12.38[a]	0.23[b]
L. S. D	18.02	1.13	34.65	1.84	0.09

Means between columns sharing the same letters are not significantly, at P<0.05.

of *Metapenaeus stebbingi*, whereas *Paphia undulata* had the minimum concentration of Cu whereas *Portunus pelagicus* had the lowest level of Hg.

No significant differences were recorded between Cd concentrations of (*Paphia undulata* and *Portunus pelagicus*) and (*Metapenaeus stebbingi* and *Portunus pelagicus*). Also, the same trend was observed between Pb and Hg concentrations of *Metapenaeus stebbingi* and *Portunus pelagicus* and between As concentrations of *Paphia undulata* and *Portunus pelagicus*. Similar trends were found by Telb (1981) who reported that the accumulation of Hg was as follow: grooved carpet shell > deep-water shrimp > blue crab.

Aboul-Dahab *et al*. (1986) showed that the concentrations of Hg in crab and shrimp obtained from Alexandria coast, Egypt varied from 0.068-0.325 and 0.029-0.314 µg/g (wet wt.), respectively.

Soliman (2006) found that accumulation of Hg in shrimp was higher than in crab, while Cd content in crab was higher than in shrimp. These results are in a good agreement with results obtained in the present study.

Except Pb concentrations of the all studied shellfish species, Cu concentrations of *Portunus pelagicus* obtained during the winter season from Abu-Qir Bay and from El-Max during the autumn season, Cu concentrations of *Donax trunculus* obtained during the winter season, As concentrations of *Paphia undulata* obtained during the summer season from Abu-Qir Bay, As concentrations of *Portunus pelagicus* obtained from El-Max during summer and autumn seasons and from Abu-Qir Bay during summer, autumn and winter seasons and *Donax trunculus* obtained from Edkou beach during autumn and winter seasons, all concentrations of Cu, Cd, , As and Hg of the four studied shellfish species were below the maximum permissiable levels reported by the different regulatory agencies FAO(1992), WHO(1996), FDA(2001) and EOSQC (1993).

As a conclusion, of the present study it can be concluded that the overall mean values of the studied heavy metals of the edible portions of the four different shellfish species collected from the different sampling areas can be arranged in a descending order as follows: Pb > Cu > As > Cd > Hg.

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تقدير بعض المعادن الثقيلة فى بعض القشريات

سعدية محمد هاشم محمد، أسامة راشد أبو سماحة، على أحمد عبد النبى ، محمد خليل محمد خليل
قسم علوم وتقنية الأغذية – كلية الزراعة – الشاطبى
جامعة الأسكندرية – الأسكندرية – جمهورية مصر العربية

أجريت هذه الدراسة بهدف تقديرمتبقيات التلوث ببعض المعادن الثقيلة والتى تشمل النحاس والكادميوم والرصاص والزرنيخ و الزئبق فى أصناف معينة من اللافقاريات البحرية المصرية الشائعة وهى الجندوفلى والجمبرى والكابوريا والتى تم جمعها من منطقتى صيد مختلفة وهما خليج المكس وخليج أبي قير وذلك خلال المواسم الأربعة فى الفترة من فبراير الى نوفمبر لعام ٢٠٠٩ وأم الخلول التى تم جمعها من مناطق صيدها الشائعة بإدكو.

أثبتت الدراسة أنه ماعدا مستويات الرصاص فى الأنواع تحت الدراسة؛ مستويات النحاس فى عينات الكابوريا التى تم جمعها من أبي قير خلال موسم الشتاء ومن المكس خلال موسم الخريف وأم الخلول التى تم جمعها من شاطىء ادكو خلال موسم الشتاء؛ مستويات الزرنيخ فى عينة الجندوفلى التى تم جمعها خلال موسم الصيف من خليج أبي قير ومستويات الزرنيخ فى عينات الكابوريا التى تم جمعها من منطقة المكس خلال موسم الصيف و الخريف و من أبي قير خلال موسم الصيف و الخريف و الشتاء وأم الخلول التى تم جمعها من شاطى ادكو خلال موسم الخريف و الشتاء ، فان كل مستويات النحاس والكادميوم و الزرنيخ و الزئبق فى الأنواع موضع الدراسة كانت أقل من الحدود المسموح بها بواسطة بعض الهيئات التشريعية مثل FAO؛ WHO وهيئة المواصفات ومراقبة الجودة المصرية.

وأشارت نتائج تحليل التباين في مستويات المعادن التى تم تقديرها ومدى تأثرها بكل من منطقة الصيد؛ موسم الصيد والنوع وتفاعلها من الدرجة الأولى (منطقة الصيد× موسم الصيد ; منطقة الصيد ×النوع و موسم الصيد × النوع) أظهرت مستويات الرصاص و الزرنيخ والزئبق أختلافات كبيرة، في حين أظهرت مستويات النحاس والكادميوم عدم وجود فروق ذات دلالة إحصائية بين المواقع (المكس وخليج أبي قير). وأظهرت جميع مستويات المعادن الثقيلة فروقاً ذات دلالة إحصائية بين فصول السنة ، باستثناء مستويات النحاس و التي لم تظهر أي اختلافات جوهرية. وأظهرت جميع مستويات المعادن التى تم تقديرها فروقا ذات دلالة إحصائية بين الأنواع المختلفة.

كذلك لم تظهربيانات تحليل التباين ANOVA أي تفاعل جوهرى بين (منطقة الصيد× موسم الصيد) ; (منطقة الصيد ×النوع) و (موسم الصيد × النوع) بالنسبة لمستويات المعادن التى تم تقديرها، باستثناء محتوى الزرنيخ في التفاعل (موسم الصيد × النوع).