EGYPTIAN JOURNAL OF AQUATIC RESEARCH 1110-0354
VOL. 31, NO.1 2005.

ACCUMULATION PATTERNS OF HEAVY METALS IN VENUS CLAMS, *PAPHIA UNDULATA* (BORN, 1780) AND *GAFRARIUM PECTINATUM* (LINNAEUS, 1758), FROM LAKE TIMSAH, SUEZ CANAL, EGYPT

KHALID M. EL-MOSELHY AND MOHAMED H. YASSIEN

National Institute of Oceanography and Fisheries, Suez

Key words: Lake Timsah, bivalves, clams, heavy metals.

ABSTRACT

The concentrations of Cd, Pb, Cu, Zn, Ni, Co, Cr, Mn and Fe in the soft tissue of bivalves *Paphia undulata* and *Gafrarium pectinatum* from Lake Timsah, Suez Canal, were determined by flame atomic absorption spectrophotometer technique. *P. undulata* showed apparent seasonal variations of metals accumulation with maximum concentrations during spring-summer months. Estimation of concentrations factor (CF) for the studied metals recorded high accumulation rates of Mn and Fe. Two-way ANOVA was conducted to evaluate the effect of inter-species and inter-size on the variability of bivalve heavy metal concentrations. ANOVA data demonstrated significant variations for the most studied metals. Correlation coefficient were calculated to study the length-metal and inter-elemental relationships. Length-metal relationships stated that Co, Cr and Mn in *P. undulata* and Cu, Zn, Ni and Co in *G. pectinatum* were independent of size, while the other metals gave significant negative correlation (at $p < 0.05$) with shell-length. The present work concluded different factors affecting the accumulation of metals in the bivalve species such as ambient concentrations and seasonal variations of metals, growth rate, uptake and excretion rates of metals and biological and gonadal development of animals.

INTRODUCTION

The Venus clams *P. undulata* and *G. pectinatum* of the family Veneridae are the most popular edible clams and represent an important bivalve fishery in Lake Timsah, where it consumed locally in Egypt and had exported to Italy. Bivalves are extensively used in monitoring programs in the marine environment due to their ability to concentrate pollutants to several orders of magnitude above ambient levels in sea water (Al-Madfa *et al.*, 1998; Domouhtsidou and Dimitriadis, 2000; Chase *et al.*, 2001; Gutierrez-Galindo and Munoz-Barbosa, 2001 and Lionetto *et al.*, 2001). According to Phillips and Rainbow (1993) an ideal biomonitor should fulfill several requisites: should be sessile or sedentary in order to be representative of the study area; should be abundant in study areas, easy to identify and sample at all times of year, and should have sufficient tissue for analysis of the contaminant of interest; should be hardy, tolerating wide ranges of contaminant concentration, thereby permitting the design of transplant experiments and laboratory studies of contaminant kinetics; and should be strong accumulators of the relevant trace metal.

Factors known to influence metal concentrations and accumulation in these organisms include metal bioavailability, season of sampling, hydrodynamics of the environment, size, sex, and changes in tissue composition and reproductive cycle (Boyden and Phillips, 1981). Seasonal variations have been related to a great extent to seasonal changes in flesh weight during the development of gonadic tissues (Joiris *et al.*, 1998, 2000). Element concentrations in molluscs at the same location differ between different species and individuals due to species-specific ability/capacity to regulate or accumulate trace metals (Reinfelder *et al.*, 1997; Otchere *et al.*, 2003). Different animals in the same community at the same trophic level could accumulate pollutants differently due to differences in habitat/niche's physical and chemical properties.

Regarding heavy metals, various processes influenced by anthropogenic activities may contribute to increase concentrations in natural waters (Singh and Steinnes 1994): (1) run-off from agricultural and urban areas, (2) discharges from mining, factories and municipal sewer systems, (3) leaching from dumps and former industrial sites, (4) atmospheric deposition. Metal pollution resulting from industrial or recreational activities is becoming an important issue as well. Because of their potentially detrimental effects on human health, the presence of heavy metals can limit the quantity of bivalves humans can consume. They can also affect the bivalves directly by killing their larvae or damaging shell growth, thus affecting their quantity and quality.

Some studies were conducted to measure the concentrations of heavy metals in bivalves and other molluscs species from Lake Timsah (Mourad, 1996; Attwa, 1997 and Abd El-Azim, 2002) The purpose of the present study was to evaluate the concentration of nine heavy metals (Cd, Pb, Cu, Zn, Ni, Co, Cr, Mn and Fe) in two popular edible bivalve species, *Paphia undulata* and *Gafrarium pectinatum* collected from Lake Timsah. In addition to study the factors affecting the accumulation rate of metals in their tissue.

Study area:

Lake Timsah, is a small and shallow lake, lies on the Suez Canal at mid way between Port Said and Suez. It lies between 30^o 33' and 30^o 35' N latitude and 30^o 16' and 30^o 19' E longitude. The region can be distinctly divided into three basins: Lake Timsah, the western lagoon and the Suez Canal pathway (Fig. 1). The lake plays an important role in most of the activities in Ismailia City, such as tourism, fisheries, navigation, etc. Lake Timsah has nearly a triangular shape with elongated sides extending roughly East-West. It has a surface area of about 8 km^2 and containing about $34x10^6$ m^3 of water and an average depth of 4-5 m (ETPS, 1995). The shores of the lake are sandy, with only restricted hard bottom of beach-rock type. It has salinity stratification where it receives brackish water from the western lagoon overtopping its high saline water. Lake Timsah is significantly polluted; the sources of pollution are essentially the raw sewage from the city network, industrial pollution from shore-line workshops, domestic sewage from unconnected areas adjoining the shore, agricultural drainage water, and possibly marine pollution.

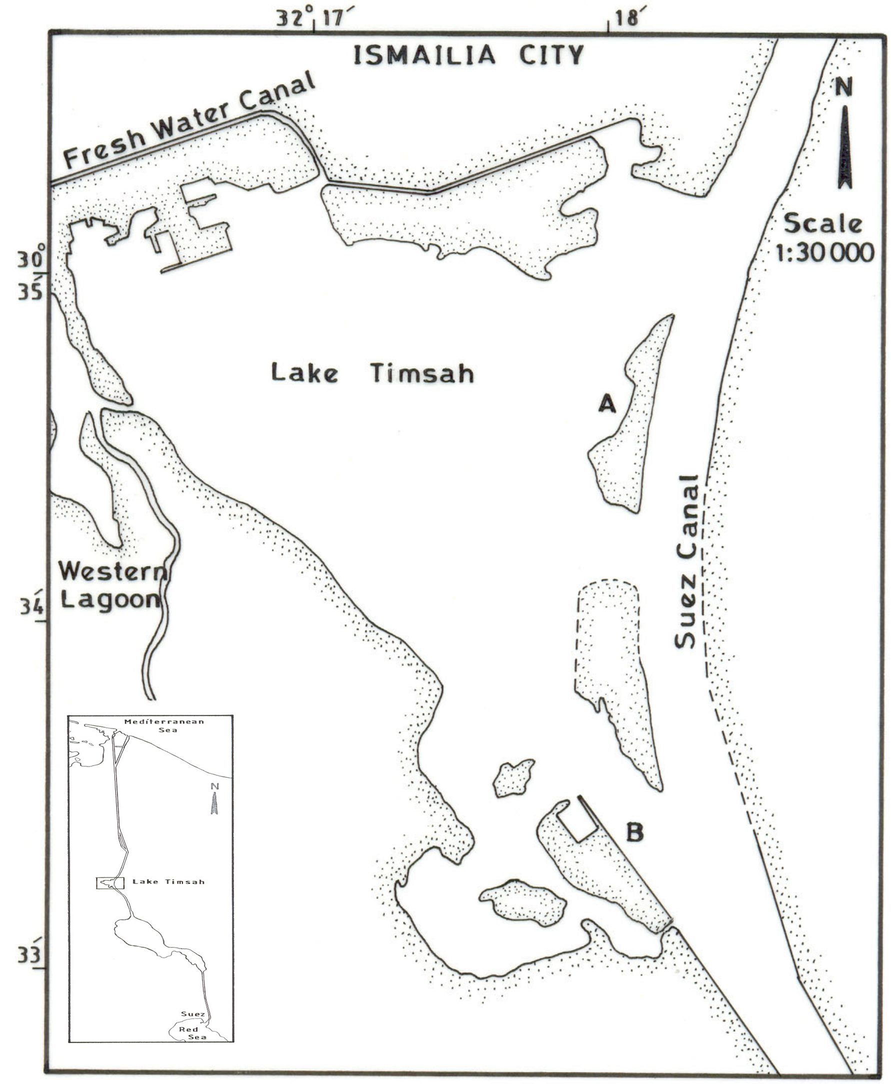

Fig. (1): Map of Lake Timsah showing the sampling sites of bivalve species: (A) ***Paphia undulata*** **and (B)** ***Gafrarium pectinatum***

MATERIALS AND METHODS

Determination of heavy metals levels:

Samples of the two clams species, *P. undulata* and *G. pectinatum*, were randomly collected monthly from Lake Timsah during the period of October 2002 – September 2003 (Fig. 1). Shell length and height were measured to the nearest 0.1 mm using a vernier caliper, then each specimen was weighed in grams (total wet weight) (Table 1). The preparation of samples to determine concentration of heavy metals was carried out according to FAO (1976). Soft tissue of the animals were separated from the shells; weighed and digested using AR conc. HNO_3 in Teflon digestion vessels. Wet digested samples were diluted with deionized distilled water and analyzed by flame atomic absorption spectrophotometer, Perkin Elmer Model Aanalyst 100. The obtained data were expressed as µg/g wet weight.

Reference material (non-defatted lobster hepatopancreas – LUTS-1) was used to check method accuracy. The recovery values of metals analysis were between 70% and 95%. As well as, the precision of analytical method was checked by replicate measurements for the studied metals in a sample of marine organism. The obtained results showed precision of 4.7 – 11.5 % for all studied metals.

Statistical analysis:

In addition to determine the level of metals in the organisms tissue, the concentration factors (CF) were calculated for the selected metals in the studied bivalves according to formula:

$$CF = C_x / C_w$$

Where: C_x and C_w are the mean concentrations of metals in the organism and in surrounding water, respectively.

Two-way analysis of variance (ANOVA) was utilized to investigate the effects of species and size (shell-length) on the variations in metal concentrations in the organisms studied. Length-metal and inter-elemental relationships were performed through the correlation matrix. All statistical analyses of data were conducted using computer program: STATISTICA for windows (Release 4.5, Copyright© StatSoft, Inc. 1993).

Table (1): Range, mean and standard deviation of shell length, height and total weight of the two bivalve species collected from Lake Timsah.

Species	No.	Shell length	Shell height	Total weight
Paphia undulata	185	2.30 - 5.97 (4.59 ± 0.63)	1.40 – 3.36 (2.58 ± 0.35)	1.26 – 20.97 (9.83 ± 4.19)
Gafrarium pectinatum	137	2.60 – 5.79 (3.96 ± 0.68)	1.89 – 3.98 (2.81 ±0.46)	3.41 – 29.65 (10.98 ± 5.44)

RESULTS AND DISCUSSION

I. Levels of heavy metals and Inter-species variations:

Temporal variations of the concentration of some heavy metals (Cd, Pb, Cu, Zn, Ni, Co, Cr, Mn and Fe) in the soft tissue of clams *P.undulata* and *G. pectinatum* are tabulated in Tables (2 and 3). It is obviously clear that, most metals in *P. undulata* were highly accumulated (0.36 µg Cd/g, 0.78 µg Pb/g, 1.64 µg Cu/g, 12.66 µg Zn/g, 1.37 µg Ni/g and 0.33 µg Cr/g) during June except Co (2.04 µg/g) was in May, Mn (5.28 µg/g) in August and Fe (153.66 µg/g) in September. In contrast the lowest values were found in the cold months (February and January) for most metals except Cd (August) and Cr (July). In respect to *G. pectinatum*, the lowest and highest values of different metals were fluctuated between the different months of the study period with opaque pattern of accumulation, and gave highest values of 0.63 µg Cd/g, 1.08 µg Pb/g, 2.47 µg Cu/g, 12.47 µg Zn/g, 1.83 µg Ni/g, 1.00 µg Co/g, 0.40 µg Cr/g, 105.30 µg Mn/g and 96.07 µg Fe/g. Attwa (1997) studied the concentration of heavy metals in *Ruditapes decussata* from Lake Timsah and found that Cd, Pb, Cu, Zn, Mn and Fe were highly accumulated in summer season. Under laboratory conditions, sandy-bottom bivalves *Donacilla cornea* showed significant difference in accumulation of copper at 2 different temperature (18 and 25 ^{o}C) with high accumulation rate at the highest temperature (Regoli, *et al*., 1991).

Seasonal differences in metals accumulation, which have been also reported in bivalve from other regions (Bryan, 1973; Fowler and Oregioni, 1976 and Szefer, *et al*., 1999a), may be related to factors such as food supply for the mollusc populations and/or runoff of particulate metal to the coastal waters of the lagoon. As well as, these seasonal variations have been related to a great extent to seasonal changes in flesh weight during development of gonadic tissues (Cossa and Rondeau, 1985; Joiris *et al*., 1998; Otchere *et al*., 2000, 2003).

Annual means and CF of the different metals in the two bivalves species are shown in Table (4). According to the annual means, the order of metals accumulation was as follow: Fe > Zn > Mn > Cu > Ni > Co > Pb > Cd > Cr and Mn > Fe > Zn > Cu > Ni > Pb > Co > Cd > Cr for *P. undulata* and *G. pectinatum*, respectively. These patterns of magnitude were changed when the order carried out according to the CF for each species, which were Mn > Fe > Cd > Co > Cu > Ni > Cr > Zn > Pb and Mn > Fe > Cu > Ni > Cd > Co > Cr > Pb > Zn, respectively. Generally, Mn and Fe showed high accumulation rates in the soft tissue of the studied bivalve species. In contrast, Zn moved to the end of the accumulation pattern (CF) comparing to its location in the concentration pattern. This phenomenon indicated that the studied species have a bioavailability to accumulate Mn and Fe from the surrounding medium greater than other metals.

Two-way ANOVA data ($p < 0.001$ and 0.01) clearly indicate significant inter-species variations in the accumulation of Pb, Cu, Ni, Mn and Fe. While, no significant differences were recorded for Cd, Zn, Co and Cr (Table, 5). This result revealed ability of one species to accumulate metals more than the other. *G. pectinatum* accumulate higher values of Pb, Cu, Ni and Mn (0.67, 1.63, 1.06 and 73.46 µg/g wet wt., respectively) than those found in *P. undulata* which shows high value of Fe only (69.29 µg/g wet wt.). Szefer *et al*. (1999b) recorded significant inter-species variation in metals accumulation in the soft tissue of molluscs species collected from the Gulf of Aden, Yemen.

Table (2): Range, mean and standard deviation of heavy metals (µg/g wet wt.) in *Paphia undulata* collected from Lake Timsah.

Month	Cd	Pb	Cu	Zn	Ni	Co	Cr	Mn	Fe
October, 2002	0.01 – 0.33	0.16 – 0.84	0.46 – 1.16	4.61 – 12.93	0.28 – 1.23	0.17 – 1.05	0.04 – 0.18	0.62 – 7.84	32.32 – 68.63
	0.18 ± 0.11	0.52 ± 0.21	0.75 ± 0.23	7.31 ± 2.10	0.49 ± 0.23	0.62 ± 0.28	0.10 ± 0.04	2.61 ± 2.03	53.07 ± 11.70
November	0.11 – 0.23	0.07 – 0.98	0.54 – 1.15	4.63 – 8.56	0.24 – 1.23	0.13 – 0.51	0.01 – 0.56	0.58 – 3.06	17.47 – 114.7
	0.16 ± 0.03	0.46 ± 0.24	0.75 ± 0.15	6.47 ± 1.36	0.46 ± 0.24	0.32 ± 0.12	0.13 ± 0.14	1.74 ± 0.76	59.75 ± 27.67
December	0.08 – 0.27	0.24 – 0.74	0.91 – 1.78	8.14 – 13.78	0.46 – 1.06	0.21 – 0.43	0.13 – 0.35	2.12 – 4.30	48.12 – 88.28
	0.19 ± 0.05	0.50 ± 0.16	1.33 ± 0.23	10.56 ± 1.66	0.62 ± 0.15	0.33 ± 0.07	0.27 ± 0.07	3.10 ± 0.67	67.52 ± 13.15
January, 2003	0.03 – 0.29	0.13 – 0.84	0.70 – 2.43	6.77 – 12.31	0.35 – 1.53	0.02 – 0.46	0.07 – 0.63	0.57 – 4.16	15.08 – 239.7
	0.16 ± 0.08	0.40 ± 0.17	1.11 ± 0.40	9.35 ± 1.51	0.73 ± 0.29	0.21 ± 0.12	0.18 ± 0.13	1.53 ± 0.81	54.47 ± 52.58
Febuary	0.08 – 0.23	0.04 – 0.53	0.39 – 0.76	4.85 – 8.04	0.20 – 0.58	0.10 – 0.33	0.02 - 0.51	0.68 – 1.97	16.05 – 59.13
	0.16 ± 0.05	0.23 ± 0.17	0.51 ± 0.11	6.22 ± 0.89	0.36 ± 0.11	0.23 ± 0.07	0.15 ± 0.14	1.05 ± 0.39	30.31 ± 12.93
March	0.07 – 0.23	0.23 – 1.05	0.41 – 1.07	4.56 – 11.84	0.31 – 0.81	0.24 – 0.50	0.04 – 0.48	0.86 – 3.77	21.41 – 100.5
	0.13 ± 0.05	0.47 ± 0.20	0.80 ± 0.18	7.27 ± 1.80	0.50 ± 0.15	0.35 ± 0.08	0.18 ± 0.11	1.68 ± 0.72	54.09 ± 23.22
April	0.05 – 0.22	0.04 – 0.77	0.83 – 2.39	4.18 – 25.29	0.38 – 1.35	0.31 – 0.66	0.02 – 0.29	1.11 – 3.87	39.13 – 107.2
	0.13 ± 0.05	0.49 ± 0.23	1.33 ± 0.36	8.78 ± 5.14	0.63 ± 0.24	0.44 ± 0.11	0.12 ± 0.07	2.02 ± 0.83	72.84 ± 18.99
May	0.21 – 0.63	0.06 – 1.91	1.09 – 1.91	8.26 – 12.89	0.53 – 1.64	1.28 – 3.27	0.08 – 0.52	1.35 – 4.21	42.71 – 174.4
	0.36 ± 0.11	0.65 ± 0.39	1.44 ± 0.26	10.26 ± 1.40	1.25 ± 0.34	2.04 ± 0.73	0.32 ± 0.11	2.90 ± 0.79	103.5 ± 30.51
June	0.21 – 0.65	0.30 – 1.25	1.35 – 2.28	9.31 – 17.11	0.64 – 2.27	0.44 – 1.28	0.13 – 0.76	2.68 – 6.41	63.50 – 129.2
	0.36 ± 0.13	0.78 ± 0.23	1.64 ± 0.25	12.66 ± 2.08	1.37 ± 0.48	0.75 ± 0.25	0.33 ± 0.16	4.36 ± 1.20	92.18 ± 21.59
July	0.06 – 0.28	0.18 – 0.69	0.53 – 1.11	6.53 – 12.65	0.26 – 0.85	0.31 – 0.60	0.01 – 0.24	0.70 – 2.61	22.90 – 78.70
	0.15 ± 0.06	0.35 ± 0.13	0.78 ± 0.15	8.78 ± 1.61	0.51 ± 0.17	0.46 ± 0.09	0.08 ± 0.07	1.31 ± 0.55	44.31 ± 19.25
August	0.02 – 0.15	0.06 – 0.65	0.54 – 1.22	6.70 – 15.95	0.52 – 0.95	0.19 – 0.37	0.01 – 0.34	2.23 – 13.45	23.14 – 76.44
	0.08 ± 0.04	0.29 ± 0.16	0.83 ± 0.20	9.40 ± 2.35	0.69 ± 0.12	0.27 ± 0.05	0.09 ± 0.08	5.28 ± 3.16	45.76 ± 14.83
September	0.13 – 0.33	0.18 – 1.05	0.53 – 0.96	5.92 – 10.03	0.38 – 1.22	0.37 – 0.85	0.06 – 049	1.33 – 4.02	94.90 – 246.5
	0.22 ± 0.06	0.51 ± 0.26	0.70 ± 0.11	7.44 ± 1.11	0.78 ± 0.24	0.59 ± 0.11	0.17 ± 0.13	2.56 ± 0.76	153.7 ± 49.33

Table (3): Range, mean and standard deviation of heavy metals (µg/g wet wt.) in *Gafrarium pectinatum* collected from Lake Timsah.

Month	Cd	Pb	Cu	Zn	Ni	Co	Cr	Mn	Fe
October 2002	0.05 – 0.90	0.42 – 2.43	0.63 – 3.61	5.15 – 14.18	0.25 – 1.22	0.43 – 1.90	0.003 – 0.17	16.74 – 84.50	31.08 – 99.44
	0.23 ± 0.23	1.01 ± 0.66	1.75 ± 0.93	8.70 ± 2.80	0.77 ± 0.33	0.89 ± 0.48	0.07 ± 0.05	50.50 ± 22.44	52.85 ± 21.16
November	0.23 – 1.31	0.36 – 1.47	0.73 – 3.90	4.24 – 10.03	0.83 – 2.90	0.42 – 1.27	0.09 – 0.26	46.01 – 164.5	36.00 – 89.32
	0.63 ± 0.31	0.74 ± 0.37	1.62 ± 0.81	7.48 ± 1.69	1.52 ± 0.67	0.71 ± 0.23	0.16 ± 0.04	82.39 ± 31.92	52.66 ± 16.04
January 2003	0.17	0.49	1.33	6.24	0.8	0.51	0.12	105.3	45.11
March	0.10 – 0.23	0.24 – 1.01	0.90 – 2.05	7.13 – 12.70	0.52 – 1.45	0.30 – 0.72	0.11 – 0.37	10.50 – 127.2	38.70 – 147.7
	0.14 ± 0.04	0.63 ± 0.25	1.46 ± 0.37	9.19 ± 1.42	0.97 ± 0.29	0.46 ± 0.10	0.25 ± 0.08	62.79 ± 28.17	86.07 ± 34.60
April	0.09 – 0.28	0.38 – 1.09	1.24 – 2.32	7.95 – 18.44	0.45 – 1.00	0.22 – 0.67	0.09 – 0.40	21.42 – 236.9	27.15 – 109.1
	0.16 ± 0.05	0.70 ± 0.18	1.73 ± 0.28	12.47 ± 3.65	0.77 ± 0.15	0.46 ± 0.12	0.20 ± 0.06	53.10 ± 49.08	57.30 ± 22.03
May	0.12 – 0.31	0.28 – 0.95	1.46 – 6.13	6.08 – 15.67	1.10 – 3.00	0.71 – 1.34	0.16 – 0.38	46.99 – 110.9	32.62 – 107.0
	0.17 ± 0.05	0.52 ± 0.18	2.47 ± 1.17	10.53 ± 2.86	1.83 ± 0.51	1.00 ± 0.23	0.26 ± 0.07	70.84 ± 16.08	73.18 ± 24.72
June	0.06 – 0.28	0.57 – 1.70	1.23 – 2.51	5.24 – 17.68	0.78 – 2.06	0.27 – 0.68	0.07 – 0.26	29.08 – 109.9	22.13 – 66.01
	0.15 ± 0.06	1.08 ± 0.34	1.90 ± 0.39	9.94 ± 4.50	1.33 ± 0.34	0.52 ± 0.10	0.15 ± 0.05	54.64 ± 22.22	44.69 ± 11.88
July	0.05 – 0.29	0.35 – 1.04	0.88 – 3.26	6.58 – 18.98	0.38 – 1.70	0.24 – 0.81	0.09 – 0.76	15.81 – 139.5	30.62 – 77.58
	0.13 ± 0.07	0.60 ± 0.19	1.73 ± 0.68	10.07 ± 3.32	0.92 ± 0.38	0.44 ± 0.17	0.40 ± 0.21	75.85 ± 38.27	47.01 ± 14.58
August	0.02 – 0.20	0.16 – 0.64	0.86 – 3.59	5.14 – 10.51	0.46 – 1.35	0.07 – 0.93	0.01 – 0.13	50.01 – 147.1	25.41 – 76.77
	0.10 ± 0.06	0.35 ± 0.15	1.51 ± 0.80	7.09 ± 1.95	0.73 ± 0.26	0.26 ± 0.25	0.08 ± 0.04	93.04 ± 26.54	41.44 ± 15.67
September	0.09 – 0.68	0.23 – 1.21	0.49 – 1.17	5.00 – 11.03	0.71 – 1.12	0.48 – 0.99	0.004 – 0.29	46.90 – 140.6	42.32 – 85.74
	0.29 ± 0.16	0.62 ± 0.28	0.81 ± 0.20	7.18 ± 1.93	0.93 ± 0.12	0.66 ± 0.12	0.14 ± 0.07	86.14 ± 23.40	64.01 ± 13.14

Table (4): Concentration factors (C.F.) of heavy metals in the soft tissues of *Paphia undulata* and *Gafrarium pectinatum* collected from Lake Timsah.

Metal	Ann. mean of metals conc. in water (µg/l) *	*Paphia undulata*		*Gafrarium pectinatum*	
		Ann. mean of metals conc. in animals	C.F.	Ann. mean of metals conc. in animals	C.F.
Cd	0.156	0.19	1217.9	0.22	1410
Pb	0.971	0.47	484	0.67	690
Cu	0.941	1	1062.7	1.63	1732.2
Zn	15.688	8.71	555.2	8.89	566.7
Ni	0.727	0.7	962.9	1.06	1458
Co	0.492	0.55	1117.9	0.59	1199.2
Cr	0.214	0.18	841.1	0.18	841.1
Mn	0.197	2.51	12741.1	73.46	372893.4
Fe	7.688	69.29	9012.7	56.43	7340

* Unpublished data.

Table (5): Effect of inter-species and inter-size on the variability of bivalve heavy metals concentrations (µg/g wet wt.) in view of analysis of variances (ANOVA).

Effect	Cd		Pb		Cu		Zn		Ni		Co		Cr		Mn		Fe	
	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Species	1	3.57	1	43.44***	1	99.77***	1	3.84	1	61.44***	1	1.44	1	2.86	1	724.2***	1	7.91**
Size [a]	3	0.67	3	6.97***	3	6.48***	3	6.35***	3	3.92**	3	0.82	3	2.99*	3	0.82	3	5.48**
Size [b]	3	3.38*	3	10.78***	3	1.58	3	2.53	3	0.16	3	4.34**	3	2.81*	3	3.59*	3	2.68*

[a] *Paphia undulata* [b] *Gafrarium pectinatum*

* significant at $p \leq 0.05$ ** significant at $p \leq 0.01$ *** significant at $p \leq 0.001$

By comparing metals concentration in the present bivalve species with those found in the same area and other Egyptian waters (Table, 6). It can observed that, most metals are comparable to the literature levels except high concentrations of Mn (50.50 – 105.30 µg/g) in *Gafrarium pectinatum* of the present work and Zn ((67.75 – 135.50 µg/g) in *Pinctada radiata* from Suez Bay (Yassien, 1998). This variation mainly attributed to bioavailability of the different species to accumulate specific metal. In addition, the present concentrations lie in the WHO limits for safety consumption of marine organisms (FAO. 1992).

II. Size-metal relationships:

Relationships (r) between shell-length of the two bivalve species and the concentration of heavy meals are given in Table (7). It can be noticed that, all metals have negative correlations with shell-length which were significant ($p < 0.05$) for Cd, Pb, Cu, Zn, Ni and Fe (r = - 0.182, - 0.234, - 0.324, - 0.175, - 0.284 and – 0.321, respectively) in *P. undulata*, and for Cd, Pb, Cr, Mn and Fe (r = - 0.241, - 0.271, - 0.221, - 0.258 and -0.229, receptively) in *G. pectinatum*. These inverse relationships can be attributed to different reasons: 1) variation in the uptake and excretion rates of metals between small and large animals, 2) small animals have ability to eat more than the large one which lead accumulate pollutant in its body, 3) dilution of the pollutant in the tissues of the large animals. In general, it can be stated that, large individuals of the two bivalve species in the present study able to regulate the metals content in their tissues.

Yassien (2004) identified four age groups for both *P. undulata* and *G. pectinatum*. The mean shell lengths in *P. undulata* were 2.92 cm, 4.44 cm, 5.28 cm and 6.06 cm for groups I, II, III and IV, respectively, while in *G. pectinatum* it were 2.79 cm, 3.75 cm, 4.45 cm and 5.12 cm, respectively. The accumulation of heavy metals in the different length groups is illustrated in figures (2 and 3). Statistically, significant inter-size variation (ANOVA data) in the concentrations of most studied metals can be observed (Table, 5).

Based on the size dependence of metal concentrations found in this study, the metals quantified can be divided into two groups: metals showing concentrations independent of size; and metals showing decreasing concentrations with size.

Among the metals studied, only Co, Cr and Mn in *P. undulata* and Cu, Zn, Ni and Co in *G. pectinatum* seemed to be independent of size. This suggests that excretion equals uptake for these metals. Other metals in the two species exhibited decreasing concentrations with size. Differing results have been reported for the effect of size on the accumulation of metals in the molluscs species. Pb, Cu and Zn concentrations were reported to be negatively correlated with size while Cd found to be independent of size (Boyden, 1977). In the same manner, Cossa *et al*. (1980) found negative correlation between Cd, Cu and Zn with mussel size. High concentrations of Cd, Cu, Zn and Mn in the oysters, *Crassostrea gigas*, in the period of growth (length of 51 – 99 mm) than in the commercial size oysters (length of 90 – 136 mm) (Gutierrez *et al*., 1991). Harris *et al*. (1979) recorded increasing patterns for Cd and Fe with shell-size. Positive relationships for Pb and negative for Cu and Fe were observed by Popham and O'Auria (1983). Cd and Pb showed positive correlation, Cu and Fe gave negative correlation, while Zn, Co and Cr found to be independent of mussel size (Riget *et al*., 1996). Zauke *et al*. (2003) observed insignificant relationships between Cd, Pb, Cu, Zn and Ni and shell-length of *Mytilus edulis*. In contrast of the present study, Szefer *et al*. (1999b) reported that the large individuals of the molluscs species collected from the Gulf of Aden, Yemen accumulated trace metals more than the small one.

As noted above, various studies have reported contradictory results with respect to the size dependence of metal concentrations in molluscs. However, it must be pointed out that the size dependence of metal levels varies considerably among molluscs species. Different ambient concentrations of metals in the environment may influence size dependence relationships, as shown by Popham and D'Auria (1983) they found Zn concentrations to be independent of size in an uncontaminated area, but positively correlated with size in a polluted area. Strong and Luoma (1981) found that the correlation between Cu and Ag and size of the clam *Macoma balthica* were influenced by size-dependence differences and seasonal variations in growth rates, and size-dependent differences in uptake rates, which gave rise to both strongly positive and strongly negative relationships. In the present study, Lake Timsah is affected by different activities particularly anthropogenical sources, which may influence the accumulation of metals in the marine organisms. In addition to seasonal variations in the metals levels as well as the physiological and biological changes of the bivalve species during the period of the study. All these factors can be affected on the metal-size relationships.

III. Metal-metal relationships:

Table (7) shows the correlation between metals in the soft tissue of *P. undulata* and *G. pectinatum*. It is obviously observed that, all metals in *P. undulata* have significant positive correlation. While, in *G. pectinatum*, only Cd: Pb-Ni-Co, Pb: Co, Cu: Zn-Ni-Co-Fe, Zn:Cr, Ni: Co-Cr-Mn, Co:Fe, Cr: Mn-Fe, Mn: Fe showed significant positive correlation and Mn: Pb-Zn showed significant negative correlation. The variations between the two species in the inter-elemental relationships may be attributed to hat *P. undulata* was collected from the site particularly closed to the different sources of pollution in the lake which can give a chance to accumulate different metals with the same magnitude. While the other species, *G. pectinatum* was collected from the site relatively faraway from the direct effect of the pollution sources.

CONCLUSION

In summary, there are generally considerable differences in the concentrations of selected metals with respect to the species (*P. undulata* and *G. pectinatum*). Data of two-way ANOVA analysis demonstrate significant inter-species variation in the accumulation of Pb, Cu, Ni, Mn and Fe. There are seasonal variations in the metals levels especially for *P. undulata* with maximum concentrations during the worm months (spring-summer). For most studied metals, inverse significant correlations are observed with the shell-length. Two-way ANOVA data show significant inter-size variations in the levels of most metals. Co, Cr and Mn in *P. undulata* and Cu, Zn, Ni and Co in *G. pectinatum* give independent pattern of size. Inter-elemental relationships show significant position correlation for all studied metals in *P. undulata*, while some of them are in *G. pectinatum* with inverse correlation between Mn: Pn-Zn.

Finally, it can be concluded that, different factors can affect the accumulation of metals and its relationships in the studied bivalve species. Such as, land-based activities which influence the ambient concentrations of metals, seasonal variations of metals content, food supply for the species population, growth rate, uptake and excretion rates of the metals, and biological and gonadal development of the animals.

Table (6): Comparison of heavy metals (µg/g wet wt.) in bivalves of the present study to those found in other Egyptian sites.

Location (Species)	Cd	Pb	Cu	Zn	Ni	Co	Cr	Mn	Fe	References
Lake Timsah (*Gafrarium pictinatum*)	0.03-0.18	0.10-1.28	0.79-5.20	2.81-17.7						Mourad (1996)
El Mex Bay (Bivalves)	0.47	0.07-0.09	2.00-2.11	17.7-20.7				5.68-6.31	14.72-17.41	El-Rayis *et al.* (1997)
Lake Timsah (*Ruditapes decussata*)	0.02–0.09	0.06-2.29	0.23-0.071	2.09-5.73				6.97-12.84	9.98-40.92	Attwa (1997) *
Suez Bay (*Pinctada radiata*)	0.16-0.75	0.33-0.84	0.51-1.89	67.8-135.5						Yassien (1998)
Lake Timsah (*Periglyta reticulata*)	0.27	0.66	1.18	11.69	0.94	0.17	0.64	3.38	65.57	Abd El-Azim (2002)
Lake Timsah (*Paphia undulata*)	0.08-0.36	0.23-0.78	0.51-1.64	6.22-12.66	0.36-1.37	0.21-2.04	0.08-0.33	0.05-5.28	30.31-153.7	Present study
(*Gafrarium pectinatum*)	0.10-0.63	0.35-1.08	0.81-2.47	6.24-12.47	0.73-1.83	0.26-1.00	0.07-0.40	50.50-105.3	41.44-86.07	Present study
WHO limit	2	2	30	1000						FAO, 1992

* dry wt.

Table (7): Correlation matrix: length vs metal; metal vs metal in the bivalve, *Paphia undulata* and *Gafrarium pectinatum* collected from Lake Timsah.

		Length	Cd	Pb	Cu	Zn	Ni	Co	Cr	Mn	Fe
Paphia undulata	Length	1									
	Cd	- 0.182*	1								
	Pb	- 0.234*	0.428*	1							
	Cu	- 0.324*	0.525*	0.402*	1						
	Zn	- 0.175*	0.306*	0.262*	0.544*	1					
	Ni	- 0.284*	0.697*	0.368*	0.639*	0.522*	1				
	Co	-0.09	0.682*	0.350*	0.426*	0.203*	0.547*	1			
	Cr	-0.076	0.395*	0.371*	0.565*	0.416*	0.499*	0.330*	1		
	Mn	-0.067	0.156*	0.248*	0.406*	0.347*	0.463*	0.145*	0.321*	1	
	Fe	- 0.321*	0.441*	0.391*	0.379*	0.198*	0.526*	0.429*	0.452*	0.368*	1
Gafrarium pectinatum	Length	1									
	Cd	- 0.241*	1								
	Pb	- 0.271*	0.387*	1							
	Cu	0.036	-0.07	0.109	1						
	Zn	0.032	-0.115	0.11	0.333*	1					
	Ni	-0.047	0.382*	0.076	0.224*	0.065	1				
	Co	-0.073	0.431*	0.428*	0.256*	0.106	0.414*	1			
	Cr	- 0.221*	-0.06	-0.034	0.132	0.203*	0.206*	0.019	1		
	Mn	- 0.258*	0.105	- 0.188*	-0.015	- 0.210*	0.204*	-0.069	0.271*	1	
	Fe	- 0.229*	0.066	0.086	0.211*	0.119	0.116	0.303*	0.277*	0.211*	1

* Significant at $p < 0.05$ (95% confidence limit)

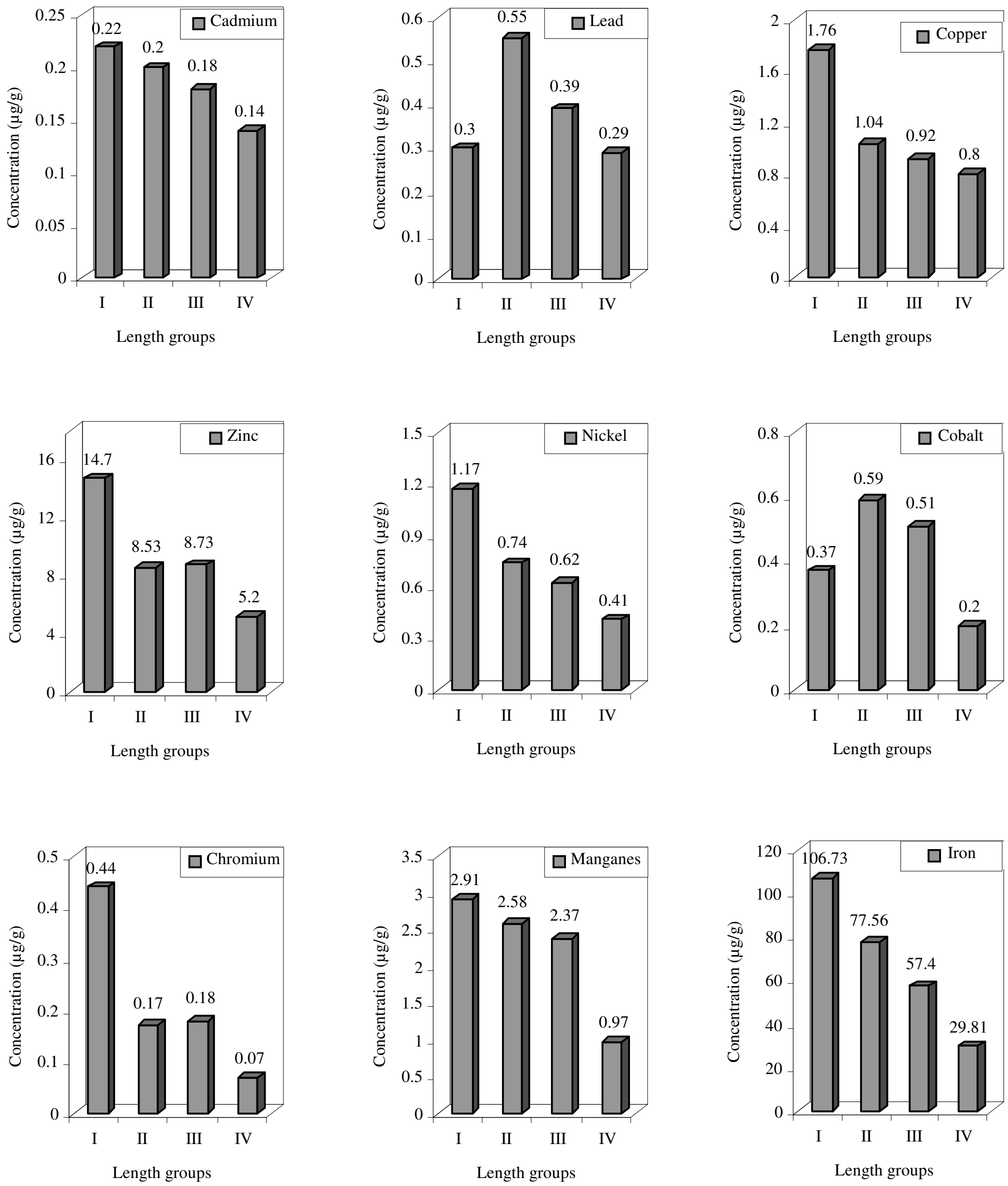

Fig. (2): Accumulation of heavy metals through the different length groups of *Paphia undulata* from Lake Timsah during 2002-2003

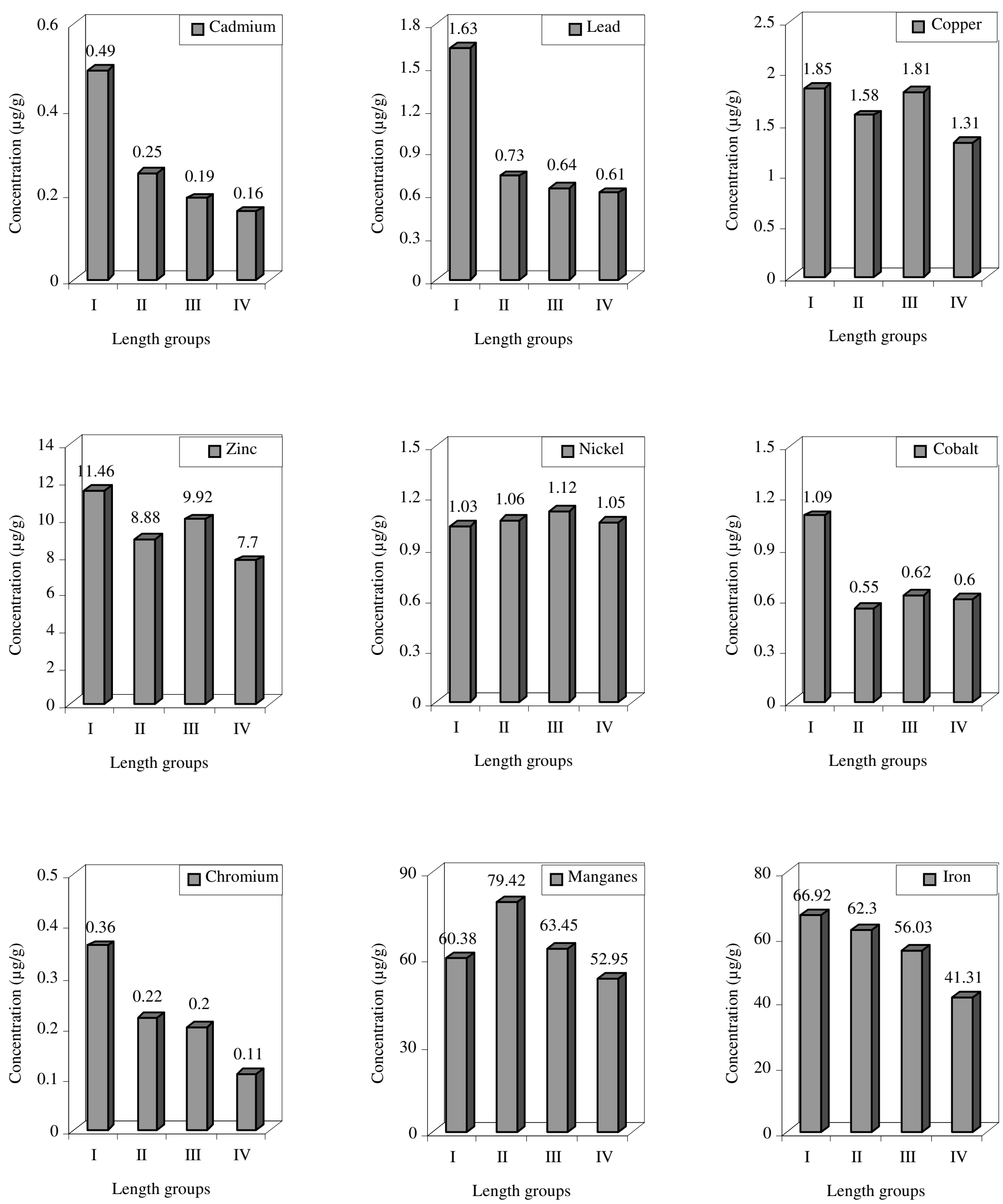

Fig. (3): Accumulation of heavy metals through the different length groups of *Gafrarium pectinatum* from Lake Timsah during 2002-2003

REFERENCES

Abdel-Azim, H. (2002): Heavy metals in Suez Canal relevant to land based sources. *Ph. D. Thesis*, Fac. Sci., El-Mansoura Univ.

Al-Mafda, H.; Abdel-Moati, M. A. R. and Al-Gimaly, F.H. (1998): *Pinctada radiata* (Pearl Oyster): A Bioindicator for Metal Pollution Monitoring in the Qatari Waters (Arabian Gulf). *Bull. Environm. Contam Toxicol*., 60: 245 - 251.

Attwa, A.H.A. (1997): Environmental study on Lake Timsah pollution by some heavy metals. *Master Degree in Environmental Science*. Institute of Environmental Studied & Research, Ain Shams Univ., Egypt. 154 pp.

Boyden, C.R. (1977): Effect of size upon metal content of shellfish. *J. Mar. Biol. Ass. U.K.*, 57: 675 - 714.

Boyden, C. R. and Phillips, D. J. H. (1981): Seasonal variation and inherent variability of trace elements in oysters and their implications for indicator studies. *Mar. Ecol. Prog. Ser*., 5: 29 - 40.

Bryan, G.W. (1973): The occurrence and seasonal variations of trace metals in the scallops *Pecten maximus* L. and *Chlamys opercularis* L.*J. Mar. Biol. Assoc*., 53:145 166.

Chase, M.E.; Jones, S.H.; Hennigar, P.; Sowles, J.; Harding, G.C.H.; Freeman, K.; Wells, P.G.; Krahforst, C.; Coombs, K.; Crawford, R.; Pederson, J. and Taylor, D. (2001): Gulfwatch: Monitoring spatial and temporal patterns of trace metal and organic contaminants in the Gulf of Maine (1991-1997) with the blue mussel, *Mytilis edulis* L. *Mar. Pollut. Bull*., 42: 491 - 505.

Cossa, D.; Bourget, E.; Pouliot, D.; Piuze, J and Chanut, J.P. (1980): Geographical and seasonal variations in the relationship between trace metal content and body weight in *Mytilus edulis*. *Mar. Biol.*,58:7-14.

Cossa, D. and Rondeau, J.G. (1985): Seasonal, geographical and size induced variability in mercury content of *Mytilus edulis* in an estuarine environment: a re-assessment of mercury pollution level in the Estuary and gulf of St. Lawreence. *Mar. Biol*., 88: 43 - 49.

Domouhtsidou, G.P. and Dimitriadis, V.K. (2000): Ultrastructural localization of heavy metals (Hg, Ag, Pb, and Cu) in gills and digestive gland of mussels, *Mytilus galloprovincialis* (L.). *Arch. Environ. Contam. Toxicol*., 38: 472 - 478.

El-Rayis, O. A.; Aboul-Dahab, O.; Halim, Y. and Riley J. P. (1997): Levels of trace metals in some food chain organisms from El Mex Bay, West of Alexandria, Egypt. *Proc. of the 7th Internat. Conf. on Environ. Prot. Is a must, 20 – 22 May, Alex., Egypt*, 26 -35.

ETPS (1995): Environmental testing of pollution status in Lake Temsah, Abu-Attwa water Reuse Center Research and training, Ismailia, Egypt.

FAO (1976): Manual of methods in aquatic environment research. Part 3, sampling and analyses of biological material, FAO Fish. Tech. Pap., *No. 158*: 124 pp.

FAO (1992): Committee for inland fisheries of Africa: Report of the third session of the working party on pollution and fisheries. *FAO Fish. Rep.*, No 471: 43p.

Fowler, S. W. and Oregioni, B. (1976): Trace metals in mussels from NW Mediterranean. *Mar. Pollut. Bull*.,7:26-29.

Gutierrez-Galindo, E. A. and Munoz-Barbosa, A. (2001): Short-term temporal variability of Ag, Cd and Cu in *Mytilus californianus* and the effectiveness of this organism as a bioindicator. *Cienc. Mar.*, 27: 269 - 288.

Gutierrez, G. E; Flores Munoz, G.; Pro Garcia, R.; Vi1laescusa Celaya, J. A. and Gonzalez Armenteros, J. A. (1991): Heavy metals in tissues and in sedimentary biodeposits of the oyster *Crassostrea gigas* from the aquaculture zone of San Quintin Bay, Baja California, Mexico. *Invest. Mar. Cicimar*., 6: 176 -186.

Harris, J. E.; Fabris, G. J.; Statham, P. J. and Tawfik, F. (1979): Biogeochemistry of selected heavy metals in emphasis in *Mytilus edulis planulatus*. *Aust. J. Mar. Freshwat. Res.*, 30: 159 – 178.

Joiris, C.R.; Azokwu, M. I.; Otchere, F.A. and Ali, I.B. (1998): Mercury in the bivalve *Anadara (Senilia) senilis* from Ghana and Nigeria. *Sci. Total Environ*., 224: 181 - 188.

Joiris, C.R.; Holsbeek, L. and Otchere, F.A. (2000): Mercury in the bivalves *Crassostrea tulipa* and *Perna perna* from Ghana. *Mar. Pollut. Bull.*, 38: 618 - 622.

Lionetto, M.G.; Giordano, M.E.; Caricato, R.; Pascariello, M.F.; Marinosci, L. and Schettino, T. (2001): Biomonitoring of heavy metal contamination along the Salento coast (Italy) by metallothionein evaluation in *Mytilus galloprovincialis* and *Mullus barbatus*. *Aqua.t Conserv.*, 11: 305 - 310.

Mourad, F.A. (1996): Heavy metals pollution in Timsah Lake. *M. Sc. Thesis*, Fac. Sci., Suez Canal Univ.

Otchere, F. A.; Joiris, C. and Holsbeek, L. (2003): Mercury in the bivalves *Anadara (Senilis) senilis, Perna perna* and *Crassostrea tulipa* from Ghana. *Sci. Total Environ.*, 304:369 - 375.

Otchere, F.A.; Joiris, C., Holsbeek, L.; Ali, I. B. and Vanderpuye, C. J. (2000): Heavy metals concentration and burden in the bivalves *Anadara* (*Senilia*) *senilis*, *Perna perna* and *Crassostrea tulipa* from Ghana; In: 11th Annual International Conference on Heavy Metals in the Environment (J. Nriagu, ed.), Contrbution number 10161. University of Michigan, School of Public Health, Ann Arbor, MI (CD-ROM).

Phillips, D. J. H. and Rainbow, P. S. (1993): Biomonitoring of trace aquatic contaminants. Elsevier, New York. 371p.

Popham, J.D. and D'Auria, J. M. (1983): Combined effect of body size, season, and location on trace element levels in mussels (*Mytilus edulis*). *Arch. Environ. Contam. Toxicol.*, 12: 1-14.

Regoli, F.; Orlando, E.; Mauri, M.; Nigro, M. and Cognetti, G. A. (1991): Heavy metal accumulation and calcium content in the bivalve *Donacilla cornea*. *Mar. Ecol. Prog. Ser.*, 74: 219 – 224.

Reinfelder, J. R.; Wang, W-X.; Luoma, S. N. and Fisher, N. S. (1997): Assimilation efficiencies and turnover rate of trace elements in marine bivalves: a comparison of oysters, clams and mussels. *Mar. Biol.*, 129: 443 - 452.

Riget, F.; Johansen, P. and Asmund, G. (1996): Influence of length on elemental concentrations in blue mussels (*Mytilus edulis*). *Mar. Pollut. Bull.*, 32 (10): 745-751.

Singh, B.R. and Steinnes, E. (1994): Soil and water contamination by heavy metals, p. 233-272. *In* R. Lal & B.A Stewart (eds.) Soil processes and water quality. Lewis, Boca Raton, Florida.

Strong, C.R. and Luoma, S.N. (1981): Variations in the correlation of body size with concentrations of Cu and Ag in the bivalve *Macoma balthica*. *Can. J. Fish. Aquat. Sci.*, 38: 1059 – 1064.

Szefer, P.; Wolowicz, M.; Kusak, A.; Deslous-paoli, J.-M.; Czarnowski, W.; Frelek, K. and Belzunce, M.-J. (1999a): Distribution of mercury and other trace metals in the cockle *Cerastoderma glaucum* from the Mediterranean Lagoon Etang de Thau. *Arch. Environ. Contam. Toxicol.*, 36: 56 – 63.

Szefer, P.; Ali, A.A.; Ba-Haroon, A.A.; Rajeh, A. A. Geldon, J. and Nabrzyski, M. (1999b): Distribution and relationships of selected trace metals in molluscs and associated sediments from the Gulf of Aden, Yemen. *Environ. Pollut.*,106:299-314.

Yassien, M.H. (1998): Biological and ecological studies on the pearl oyster; *Pinctada radiate* (Mollusca, Lamellibranchia) from the Red Sea, with special reference to its tolerance to water pollution. *Ph. D. Thesis*, Fac. Sci., Ain Shams Univ.

Yassien, M. H. (2004): Length-based growth parameter estimates of the Venus clams *Paphia undulata* (Born, 1780) and *Gafrarium pectinatum* (Linnaeus, 1758) from Timsah Lake, Suez Canal, Egypt. *J. Aquat. Biol. & Fish.* (in press).

Zauke, G.P.; Clason, B.; Savinov, V.M. and Savinova, T. (2003): Heavy Metals of Inshore Benthic Invertebrates from the Barents Sea. *The Science of the total Environment*, 306/1-3: 99 – 110.