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Heavy Metals Accumulation in Marine Edible Molluscs, Timsah Lake, Suez Canal, Egypt

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ABSTRACT

Aims: to investigate and to compare the variation of the bioaccumulation of heavy metals (Cr, Cd, Fe, Mn, Pb and Zn) by two different molluscs species and also heavy metal concentration in sea water and sediment in Timsah Lake-Suez Canal. And finally to study the correlation between metal concentrations in different size classes of each molluscan species.

Study Design: Levels of the heavy metals (Cr), (Cu), (Fe), (Mn), (Pb), and (Zn) were determined in water, sediment and soft tissues of the gastropod *Thais carinifera* and the bivalve *Venerupis aureus* in Timsah Lake- Suez Canal.

Place and Duration of Study: Marine Sciences Department, Faculty of Science, Suez Canal University, Ismailia, Marine Sciences Department, Faculty of Science, Port Said University, Egypt, in April 2013.

Methodology: Surface sediment sample (15 cm), Sample of water (0.5 m depth) and 244 molluscan specimens (65 gastropods and 179 bivalves) covering a wide size-range was collected from sampling site.

Results: The concentrations of heavy metals in water were 0.5, 0.9, 2.5, 0.22, 0.41 and 0.25 for Cr, Cu, Fe, Mn, Pb and Zn $\mu\text{g L}^{-1}$, respectively. The corresponding concentration values in the sediments 12.8, 10.2, 18.8, 14.5, 12 and 45 $\mu\text{g g}^{-1}$ dry weight for Cr, Cu, Fe, Mn, Pb and Zn, respectively. While the heavy metals concentrated in molluscs was 17.5, 17.4, 933.5, 20.9, 30.25 and 89 ($\mu\text{g g}^{-1}$ dry weight) for Cr, Cu, Fe, Mn, Pb and Zn, respectively.

Conclusion: The accumulation of heavy metals is predominant in molluscs than in sediment and water. The gastropod (*t. Carinifera*) and the bivalve (*v. Aureus*) accumulate different amount of metals in their tissues. The accumulation of metals was more pronounced in *v. Aureus* than *t. Carinifera*. A positive correlation between metal concentration and size of the gastropod *t. Carinifera* was found, which means that the largest individuals contained the highest levels of metals. While metal concentrations increased with length in *v. Aureus*.

Keywords: Heavy metals; *Thais carinifera*; *Venerupis aureus*; Timsah Lake; Suez Canal.

1. INTRODUCTION

During the past decades, Suez Canal region has become a matter of growing international concern. The semi-enclosed nature of this water body, the characteristics of its current system and the increasing population density along its coastline are source of water pollution. One of the threats to the Suez Canal (and its Lakes) ecosystem is metal pollution. Trace metals originate from a number of anthropogenic and natural sources [1,2,3,4]. The heavy metal concentrations may be attributed to sewage and agriculture drainage [5]. Beside the corrosion of ship's hulls coating and antifouling paints at ships awaiting berth can cause high concentration of copper in the lake. The lake also receives effluents from the industrial complex (Ismailia free zone) which includes mainly textile and dyes factories [6]. Some trace metals can have a negative effect on aquatic organisms due to high accumulation in their body tissues [7,8] and consequently can cause a human health risk when these organisms are consumed [9,10]. Human require

varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc have been linked to human growth, development, achievement, and reproduction [11]. Excessive levels can be damaging to human. Other heavy metals such as mercury, plutonium, and lead are toxic metals that have no known vital or beneficial effect on organisms and their accumulation over time in their bodies can cause serious illness [12,8]. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Repeated long-term contact with some metals (or their compounds) may cause cancer [11].

Marine organisms such as clams, bivalves, cockles [13,14] and gastropods [14] have been used as

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bioindicators for trace metal pollution [15]. The gastropod (*Thais carinifera*) and the bivalves (*Venerupis aureus*) are common edible species of the Timsah Lake [16,17]. However, information on metal concentrations in these organisms in Timsah Lake is limited.

So, this study aims to investigate and to compare the variation of the bioaccumulation of heavy metals (Cr, Cd, Fe, Mn, Pb and Zn) by two different molluscs species and also heavy metal concentration in sea water and sediment in Timsah Lake-Suez Canal. And finally to study the correlation between metal concentrations in different size classes of each molluscan species.

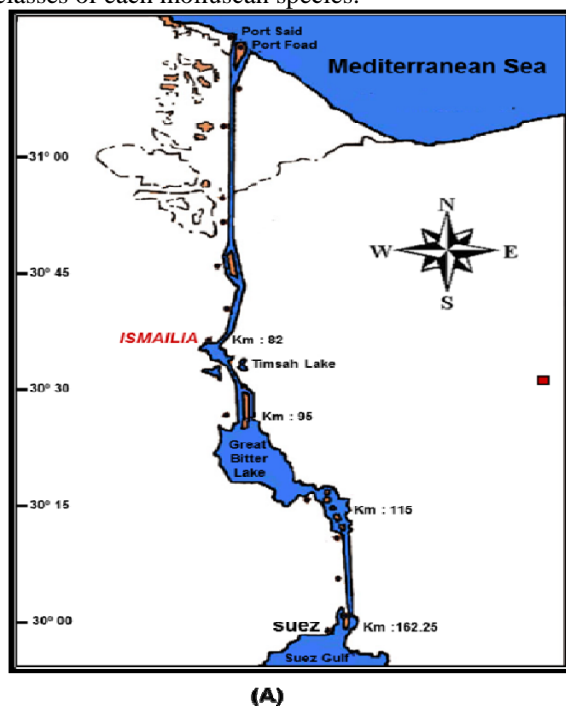


Fig 1: Map of Timsah Lake showing the sampling sites. (A) Position of Timsah Lake in the Suez Canal. (B) Sampling sites on Timsah Lake

Surface sediment sample (15 cm) was collected from sampling site using a grab sampler. Samples were stored into plastic vessels and frozen at -20°C until analysis. In the laboratory, sediment samples were defrosted at room temperature, dried in a drying oven at 40°C up to a constant weight, ground and homogenized in a mortar to fine powder. Sediment sub-sample of 0.5 g was digested with a mixture of concentrated acids (HNO_3 and HClO_4 suprapure) in glass vials for 2 hours according to the method described by [18].

Sample of water (0.5 m depth) was collected. The total metal content in the sea water was determined following the method of [19]. The metals were pre-concentrated from sea water using ammonium pyrrolidine dithiocarbamate (APDC) as chelating agent and after extraction were dissolved in methyl isobutyl ketone

2. MATERIALS AND METHODS

Timsah Lake is situated between $30^{\circ} 33' 3''$ and $30^{\circ} 35' 31''$ N and $32^{\circ} 16' 30''$ and $32^{\circ} 18' 50''$ E. It has a surface area of 15 km^2 with a depth range from the 6-13 m (Fig. 1). It receives fresh water coming from the over flow of the Ismailia fresh water canal, heavy domestic, industrial and agricultural waste water from El-Mahsama drain, Abu-Gamous and untreated sewage from El-Bahtini region [5], Land based activities and ships awaiting berth are also the main source of metal pollution.

(MIBK). The organic extract was then aspirated directly to a flame atomic absorption spectrophotometer to determine the metal concentration.

The collected molluscan samples (gastropods and bivalves) were collected in April 2013 from the fishing site of Timsah Lake. Sorting the molluscs from the sediments by sieve its mesh size was 4 mm. A total of 244 molluscan specimens (65 gastropods and 179 bivalves) covering a wide size-range were collected to prepare a pooled sample to reduce individual variations in heavy metal concentrations [20,21]. The collected gastropod species (*Thais carinifera*) belongs to family Muricidae and the bivalve species (*Venerupis aureus*) belongs to family Veneridae. The animals were pooled into size groups; four size groups were set at 5 mm intervals for *T. carinifera* (50-55 mm, 55-60 mm, 60-65 mm, and 65-70 mm) and six size

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groups were set at 5 mm intervals for *V. aureus* (25-30 mm, 30-35 mm, 35-40 mm, 40-45 mm, 45-50 mm and 50-55mm). The edible part of the meat was carefully removed by shelling the samples with a plastic knife, they were then dried in an oven to constant weight at 65°C for 72 h., homogenized [20] and ground to a fine powder in a mortar before analysis [22,23]. About 0.2 g from each sample was digested with a mixture of concentrated acids (HNO₃ and HClO₄ Suprapure, Merck) in glass vials [24]. The completely digested samples were allowed to cool at room temperature, filtered and made up to 25 ml. All digested samples were analyzed, in triplicate. Atomic absorption spectrophotometer (GBC Avanta E Atomic Absorption) was used for the determination of the heavy metals Cu, Cr, Fe, Mn, Pb and Zn.

To compare the total content of metals of the two different molluscan species, the Metal Pollution Index (MPI) was used, obtained with the equation [25,26,27] where,

$$MPI = (Cf_1, \times Cf_2, \dots Cf_n)^{1/n}$$

Cf_i = concentration for the metal i in the sample.

n = number of metals.

Bioconcentration factor was used to estimate the accumulation of heavy metals in mollusca [28] where,

$$BCF = \frac{\text{concentration biota}}{\text{Concentration water}}$$

3. RESULTS

The concentrations of heavy metals (Cu, Cr, Fe, Mn, Pb and Zn) in water, sediment and molluscs collected from Timsah Lake during 2013 are given in Table 1. It was observed that Fe attained the maximum values in the two components; water (2.5 µg L⁻¹), and molluscs (933.5 µg g⁻¹) while Zn revealed the maximum value in sediment (45 µg g⁻¹). Mn recorded the minimum value in water (0.22 µg L⁻¹), whereas, Cu recorded minimum values in sediment (10.2 µg g⁻¹) and molluscs (17.2 µg g⁻¹). The concentration of heavy metals in water could be arranged in the following sequence Fe > Cu > Cr > Pb > Zn > Mn, while the sequence in sediment was Zn > Fe > Mn > Cr > Pb > Cu and in molluscs was Fe > Zn > Pb > Mn > Cr > Cu. The results indicated that the accumulation of heavy metals is predominant in molluscs than in sediment and water (Table 1).

Table 1: Heavy metal concentrations in water sample (µg L⁻¹), sediment sample (µg g⁻¹ dry weight) and molluscan species (µg g⁻¹ dry weight) collected from Timsah Lake

Metal	Water (µg L ⁻¹)	Sediment (µg g ⁻¹)	Mollusca (µg g ⁻¹)
	Mean ± SD	Mean ± SD	Mean ± SD
Cr	0.5 ± 0.81	12.8 ± 0.76	17.5 ± 0.28
Cu	0.9 ± 1.75	10.2 ± 1.29	17.4 ± 1.17
Fe	2.5 ± 1.34	18.8 ± 1.43	933.5 ± 1.23
Mn	0.22 ± 1.89	14.5 ± 1.58	20.9 ± 0.98
Pb	0.41 ± 1.66	12 ± 1.42	30.25 ± 1.34
Zn	0.25 ± 1.48	45 ± 0.62	89 ± 1.54

The estimation of the heavy metals (Cu, Cr, Fe, Mn, Pb and Zn) within the soft tissues of the gastropod (*T. carinifera*) and the bivalve (*V. aureus*) is displayed as µg g⁻¹ in Table 2. The highest mean values of heavy metals were Fe (618.7 and 1248.3) and Zn (75.2 and 102.8) in both *T. carinifera* and *V. aureus*, respectively. On the other hand, the lowest mean value of heavy metals in *T. carinifera* was Cu while Cr represents the minimum value in *V. aureus*. Therefore, the order of metal concentrations in *T. carinifera* was Fe > Zn > Pb > Mn > Cr > Cu and in *V. aureus* was Fe > Zn > Cu > Mn > Pb > Cr.

The two molluscan species; the gastropod (*T. carinifera*) and the bivalve (*V. aureus*) accumulate different amount of metals in their tissues. Analysis of variance (one

way ANOVA) showed that the concentrations of Cr, Mn and Pb were significantly higher ($p < 0.01$) in *T. carinifera*

than in *V. aureus*. On the other hand, the metals Cu, Fe and Zn levels in *V. aureus* were significantly higher ($p < 0.01$) than in *T. carinifera*. Generally, Metal Pollution Index (MPI) showed that the accumulation of metals was slightly more pronounced in *V. aureus* (MPI = 3.355) than *T. carinifera* (MPI = 3.041).

Table 3 shows the bioaccumulation factor (BCF) to estimate the accumulation of heavy metals in the two different molluscan species. BCF in the present study ranged from 15.4 (Cu) and 300.8 (Zn) in *T. carinifera* whereas in *V. aureus* Cu attained the BCF minimum value 23.2 and Fe attained the maximum value (499.4).

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Table 2: Heavy metal concentrations and mean metal pollution index (MPI) in the two molluscan species (gastropod and bivalve)

Metal	Mollusca ($\mu\text{g g}^{-1}$)	
	Gastropod	Bivalve
	<i>T. carinifera</i>	<i>V. aureus</i>
Cr	18.3	16.7
Cu	13.9	20.9
Fe	618.7	1248.3
Mn	22.1	19.7
Pb	42.7	17.8
Zn	75.2	102.8
MPI	3.041	3.355

Table 3: Bioaccumulation factor (BCF) in the two molluscan species (gastropod and bivalve)

Metal	BCF in	BCF in
	<i>T. carinifera</i>	<i>V. aureus</i>
Cr	36.6	33.4
Cu	15.4	23.2
Fe	247.5	499.3
Mn	100.5	89.5
Pb	104.1	43.4
Zn	300.8	411.2

Table 4 shows the relationships between metal concentrations and different molluscan size groups analyzed. The gastropod *T. carinifera* ranged in size length from 50 to 70 mm (four size groups) while size of the bivalve *V. aureus* ranged from 25 to 55 mm (six size groups). Positive correlations were found between the size of *T. carinifera* and Cr, Cu, Fe, Pb and Zn ($P < 0.05$). The largest individuals contained the highest levels of metals. On the other hand, all studied metals (Cr, Cu, Fe, Mn, Pb and Zn) had positive correlations related to size in *V. aureus* ($P < 0.01$). The smaller the bivalve (size class 25 – 30 mm), the higher the heavy metal concentration (Table 4).

Table 4: The total shell length and metal levels in soft tissue of *Thais carinifera* and *Venerupis aureus* collected from the Timsah Lake

Thais carinifera							
Total length (mm)	N	Metal concentrations ($\mu\text{g g}^{-1}$ dry weight)					
Size classes		Cr	Cu	Fe	Mn	Pb	Zn
(50-55) I	7	18.2	11.3	509.0	26.3	33.0	81.0
(55-60) II	26	18.3	10.0	375.5	17.5	45.2	68.5
(60-65) III	28	18.0	17.0	577.6	22.5	45.0	62.5
(65-70) IV	4	18.5	17.5	1012.5	22.0	47.4	88.6
Venerupis aureus							
Total length (mm)	N	Metal concentrations ($\mu\text{g g}^{-1}$ dry weight)					
Size classes		Cr	Cu	Fe	Mn	Pb	Zn
(25-30) I	47	18.8	26.0	1512.5	27.3	19.1	132.3
(30-35) II	42	16.7	25.2	1502.0	18.8	18.5	112.6
(35-40) III	12	16.3	24.0	1425.5	18.8	17.2	102.8
(40-45) IV	38	15.8	25.0	1272.5	18.0	17.1	117.5
(45-50) V	32	16.2	12.5	1215.0	13.8	17.6	73.4
(50-55) VI	8	16.2	12.5	562.5	21.5	17.3	78.3

N: number of samples

4. DISCUSSION

In the present study, it was noticed that Fe is known to be the main heavy metal contaminant in Timsah Lake. Fe concentration revealed the maximum value in water ($2.5 \mu\text{g L}^{-1}$), and molluscs ($933.5 \mu\text{g g}^{-1}$). This is in agreement with [1] who study the heavy metal concentration in Timsah Lake and they found that Fe attained the maximum value in water ($1.27 \mu\text{g L}^{-1}$), and

molluscan bivalve *Ruditapes decussatus* ($961.5 \mu\text{g g}^{-1}$). Also, [29] who study the heavy metal in the same study area agree with the same result; where Fe concentration was one the highest metal concentration in water ($11.29 \mu\text{g L}^{-1}$) and the bivalve *R. decussatus* ($2182.2 \mu\text{g g}^{-1}$). [30,31] find that Fe in water attained the maximum value in Suez Canal and Suez Gulf and this in agreement with the present study.

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In sediment, Zn ($45 \mu\text{g g}^{-1}$) represents the maximum concentration of heavy metal. This agrees with that recorded by [1] who noticed that the maximum value of heavy metal in sediment of Timsah Lake was Zn ($62.7 \mu\text{g g}^{-1}$).

Many marine organisms are known to accumulate relatively high heavy metal concentrations [30]. In our study, this is particularly true for all studied metals (Cu, Cr, Fe, Mn, Pb and Zn); the accumulative indices for heavy metals in molluscan tissues with respect to sediment heavy metal concentrations are quite high. Metal biomagnification is very pronounced in molluscan species than in sediment and water.

In the current study, the greatest value of heavy metals were recorded for Fe ($618.7 \mu\text{g g}^{-1}$ and $1248.3 \mu\text{g g}^{-1}$) and Zn ($75.2 \mu\text{g g}^{-1}$ and $102.8 \mu\text{g g}^{-1}$) in gastropod (*T. carinifera*) and bivalve (*V. aureus*), respectively. This is in agreement with [29], who studied the heavy metal concentrations in gastropod (*Patella caerulea*) and bivalve (*Barbatus barbatus*) in Suez Canal.

By comparing between gastropod and bivalve species, it was clearly indicated that Cr, Mn and Pb are more concentrated in the gastropod *T. carinifera*. This is in agreement with Hamed 1996, who stated that the gastropod (*Patella caerulea*) concentrates more the same metals. In contrast, bivalve (*V. aureus*) concentrated the metals Cu, Zn and Fe than gastropod in the current work, this partially agrees with [31] who stated that Cu and Zn are more concentrated in bivalve than gastropod.

Generally, in the present work metal pollution index (MPI) of bivalve was slightly greater than that of gastropod. In contrast, [30,31] stated that the MPI of gastropod was greater than that of bivalve in Suez Canal and Suez Gulf. There are many factors effect metal pollution index, as these organisms take metals from all environmental compartments due to their habitat, their feeding habits and heavily concentrate them [32]. Moreover, these animals are sessile or sedentary, available all year long [33,34].

On studying the relationship between size and heavy metal concentration, it was observed that the largest gastropod individual (65 – 70 mm) contained the highest levels of heavy metals (Cr, Cu, Fe, Pb and Zn). This is in agreement with [13] who found a positive correlation between heavy metal concentrations and size of limpets and snails, which means that the largest individuals contained the highest levels of heavy metal concentrations.

On the other hand, heavy metal concentration is greater in small size bivalve (25 – 30 mm) while it decreased in larger size (50 – 55 mm). It concluded that, the smaller the bivalve, the higher the metal concentration.

[35,36] have established that the trace metal content of most molluscs is related to their size. [37] observed similar results with Cd, Pb and Zn in a population of snails and attributed this pattern and its variations to the metabolic activity of the animals. He suggested that the increase in metabolic rates in younger individuals may affect metal uptake and elimination differentially. [27] noticed that independence between metal concentrations and size occurs when the uptake and excretion rates of the metals balance.

The main objective of this study showed if the selected molluscan species were suitable for human consumption. Among the different metals analyzed Cr and Pb are classified as chemical hazards [38,39,40,41]. It has been estimated that the average daily human requires of Cr is $1 \mu\text{g}$ [42]. Deficiency of Cr results in impaired growth and disturbances in glucose, lipid, and protein metabolism [43]. In the present study, Cr concentrations were fluctuated between 16.6 and $18.3 \mu\text{g g}^{-1}$ dry weights. Molluscan samples exhibit a relatively higher Cr concentration than the permissible $13 \mu\text{g g}^{-1}$ [40].

Pb is the second element on the top 20 list of the most poisoning heavy metals. Its target organs are bones, brain, blood, kidneys, and thyroid gland, reproductive and cardiovascular systems [44,45]. The accumulation of Pb in molluscs under investigation ranged between 17.8 and $42.7 \mu\text{g g}^{-1}$ dry weight. These results showed very high Pb concentrations exceeding the maximum allowable levels of [38] ($0.5 \mu\text{g g}^{-1}$).

High levels of zinc cause pancreatitis, anemia, muscle pain and acute renal failure [46]. The concentration range of Zn in the studied mussels was 75.2 – $102.8 \mu\text{g /g}$ dry weight, which exceeds the allowed level of [38] ($40 \mu\text{g g}^{-1}$).

5. CONCLUSION

The accumulation of heavy metals is predominant in molluscs than in sediment and water. The gastropod (*T. carinifera*) and the bivalve (*V. aureus*) accumulate different amount of metals in their tissues. The accumulation of metals was more pronounced in *V. aureus* than *T. carinifera*. A positive correlation between metal concentration and size of the gastropod *T. carinifera* was found, which means that the largest individuals contained the highest levels of metals. While metal concentrations increased with length in *V. aureus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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