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Biomarker Approach of Freshwater Mussels *Nitia teretiuscula* and *Spathopsis rubens* (Bivalvia: Unionidae, Mutelidae) as Invertebrate Bioindicators in Monitoring Aquatic Environmental Quality

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Abstract: ***Bivalves are abundant in many types of freshwater environments and are relatively easy to collect and identify also they have been used frequently as bioindicator organisms. At metal concentrations that are within ranges common to natural waters, mussel species are generally effective bioaccumulators. Pollution of water and sediment of River Nile is a threat on survival and existence of bivalves. Concentrations of six heavy metals were analyzed in the soft parts (foot, mantle, gills and digestive tissue) of the investigated mussel species Nitia teretiuscula Philippi, 1818 and Spathopsis rubens Lamarck, 1819, as well as the Nile water and sediment samples collected from Tura (Helwan Governerate) and El-Kanater (Qalyuobia Governerate). Fe and Mn showed the highest level of metals accumulated in different soft parts of both species (g/Kg/ dry wt) collected from two localities and were ranged between 303.26-669.45, 424.8-788.7 for Fe and 156.5-592.4, 115.4-649 for Mn in soft parts of N. teretiuscula and S. rubens, respectively, collected from Tura region and 144.49-481.6, 152.47-911.69 for Fe and 263-475.3, 306-628.9 for Mn in soft parts of N. teretiuscula and S. rubens, respectively, collected from El-Kanater region. Other metals analyzed are fluctuated in their levels of accumulation. However, all metals measured in the collected tissues exceeded the permissible levels. Also, bioconcentration factor (BCF) and transfer factor (TF) were calculated. The highest BCF observed for Mn and the lowest recorded for Co measured in the digestive tissue of N. teretiuscula collected from Tura region (13381.5 g/Kg/dry wt), (4.2 g/Kg/dry wt), respectively. In addition, the TF of the sediments to tissues (g/Kg/dry wt) was greater than that of water (mg/ L) to tissues 0.2-5.7, 0.0001-0.2 in Tura and 0.2-3.4, 0.0001-0.08 in El-Kanater regions, respectively.***

Keywords: Freshwater mussels, Pollution, Heavy metals accumulation, *Nitia teretiuscula*, *Spathopsis rubens*

1. Introduction

Contamination of freshwater with a wide range of pollutants has become a matter of great concern over the last few decades [33]. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities [1, 37, 51]. Discharge of heavy metals into rivers or any aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior [2, 11]. Some heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes. Other metals, such as cadmium, mercury, and lead have no known essential role in living organisms, and are toxic even at low concentrations. While, the essential metals become toxic at high concentrations [80]. The consequence of heavy metal pollution can be hazardous to human population through food. Thus, monitoring programs and research on heavy metals in aquatic environments and their accumulation by aquatic organisms have become very vital to nature management, human consumption of these species through food chain, extinction of others, in addition to determine the most useful biomonitor species and the most polluted area [63]. Most studies that provide comparisons among taxonomic groups indicate that bioaccumulation in molluscs are greater than that in fish [3, 5, 6, 7]. The importance of molluscs as essential organisms in metal biomonitoring is widely recognized [9, 16, 17, 43, 68, 72]. Of all the possible biomonitors available for monitoring aquatic environments, bivalves fulfill most of characteristics as they are widely distributed globally, easy to handle and sessile. Also, they are filter feeders that have the ability to accumulate high metal concentrations without metabolising the metals appreciably [22, 28, 34, 61, 66] provide a time-integrated indication of environmental contamination [12, 14, 26, 70].

Freshwater bivalves in Egypt are endangered due to heavy metal pollution of the Nile River. Also, mussels are considered as a source of protein among locals. Therefore, the aim of this study was to estimate the concentration of six heavy metals in different tissues of two bivalves, *Nitia teretiuscula* and *Spathopsis rubens*, in addition, water and sediment of area under investigation, El-Kanater and Tura regions in order to estimate the quantities that enter to human body and relationships between metals in tissues and sediment.

2. Materials and Methods

2.1. Samples collection

2.1.1. Collection of mussels

Nitia teretiuscula and *Spathopsis rubens*, were collected from two localities, El-Kanater (Qalyuobia Governerate) and Tura (Helwan Governerate). Twenty samples were monthly and randomly collected from Nile River; during the period of September 2016 to August 2017. Collection of samples was carried out using a specially device net made of hard metallic frame, provided with wire and divided into squares of about 0.2 mm in diameter like a mesh. By scraping the bottom of freshwater streams with the frame and gently shoring the net from the water surface, the mud collected was washed out, leaving the mussels in the net to be picked out. Collected mussels were then transferred to Laboratory of Invertebrates, Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt; then sorted and maintained under the same conditions of food and temperature.

2.1.2. Collection of water and sediment samples

Nile water and sediment were collected in bottles from the two areas of study during the period of the current investigation and transported immediately to Micro-analytical center in Cairo University, Egypt, for determining physiochemical factors and heavy metals levels.

2.2. Determination of physiochemical factors of the Nile Water

2.2.1. Temperature

It was measured monthly at the time of sampling from the field, using a mercury thermometer graduated from 0ºC to 100ºC, by immersing it for 5 min in water at each locality and the temperature was directly recorded. In the lab, groups of 20 mussels of the collected specimens were placed in water bath at different temperatures ranging from 15 to 45ºC to record the effect of temperature on survival of studied mussels.

2.2.2. pH

It was measured by taking a sample of the Nile water from both localities (El-Kanater and Tura). pH of each sample was measured monthly for one year using a pH meter model Accumet® 925 (Fishar Scientific). The effect of pH was also observed by putting groups of 20 mussels of each species in different pH levels, adjusted by adding drops of acetic acids.

2.2.3. Water analysis

Copper (Cu), Cobalt (Co), Nickel (Ni), Manganese (Mn), Lead (Pb), and Iron (Fe) are analysed in Nile water samples collected from the two localities to determine their concentrations using atomic absorption spectrophotometer model A-Analyst 100 Perkin Elmer.

2.3 Chemical Analysis

Soft parts were excised on clean petri dishes after dissection of mussels for analysis of the heavy metals, Copper (Cu), Cobalt (Co), Nickel (Ni), Manganese (Mn), Lead (Pb), and Iron (Fe).Wet weights were measured by the method of [36,54] Tissues were dried at room temperature for one day, then dry tissues placed in 1.5 ml washed microcentrifuge tubes. 5 ml of piperidine (mole/litre) was added, and then the tubes were cooled to room temperature, followed by addition of 2 ml of 61% (V/V) $HClO_4$ to the precipitate. After 10 min., 7 ml of deionized water was added and centrifuged for one minute, at 10.000 rpm in a microcentifuge (BeckMan/Model J-2, 21). Supernatants were collected in aliquots for analysis; using an atomic absorption spectrophotometer, model A-Analyst 100 Perkin Elmer instrumentation laboratories. Bioconcentration Factor (BCF) was calculated as the ratio of concentration of metal in the tissues to the concentration in water or sediment, according to Sharma et al. [74]. Also, the transfer factor (TF) was calculated according to Kalay and Canli [37] as follows: Transfer factor (TF)=M_{tissue}/ $M_{sediment}$ or M_{water}.Where, M_{tissue}, metal accumulated within mussel's tissue, $M_{sediment}$, metal measured in sediment, and M_{water}, metal determined in water.

2.4 Statistical Analysis

It carried out using SPSS program Version 19 to determine the significance differences between mean values of the different parameters in the studied mussels. The data of two sets of sediment and water samples between the two regions analyzed by using t-test and one-way ANOVA. MANOVA test was employed to find the differences in the morphometric and ecological analysis of the different soft parts between the two species at a probability level $P>0.05$ for insignificant results and $P<0.05$ and $P<0.0001$ for significant results.

3 Results

3.1. Physiochemical factors of the Nile Water

3.1.1. Temperature (°C) and pH of the River Nile water, at Tura and El-Kanater sites (Fig. 1) during the one year of experimental period were measured monthly (Fig. 2a,b), and ranged between 16°C at winter season to 27°C in summer season in both regions. Also, pH measured found to be alkaline ranging from 8.1-8.53 in Tura and 7.3-7.85 in El-Kanater.

Figure 1: Map of Egypt showing the studying areas.

3.1.2. Effect of Temperature on mussel species survival

It was found that mussels under investigation could withstand various temperatures ranging from 15-40°C, with optimum temperatures of 25-30°C but at temperature greater than 40-45°C, the studied mussels started to die and above 45°C complete death occurred. At15°C, 95% of the mussel species survived. While, 100% survived at 20°C, 25°C and 30°C. But, 85% survived at 35°C and 75 % remained alive at 40°C. Above 40°C all mussels died (Fig. 2a).

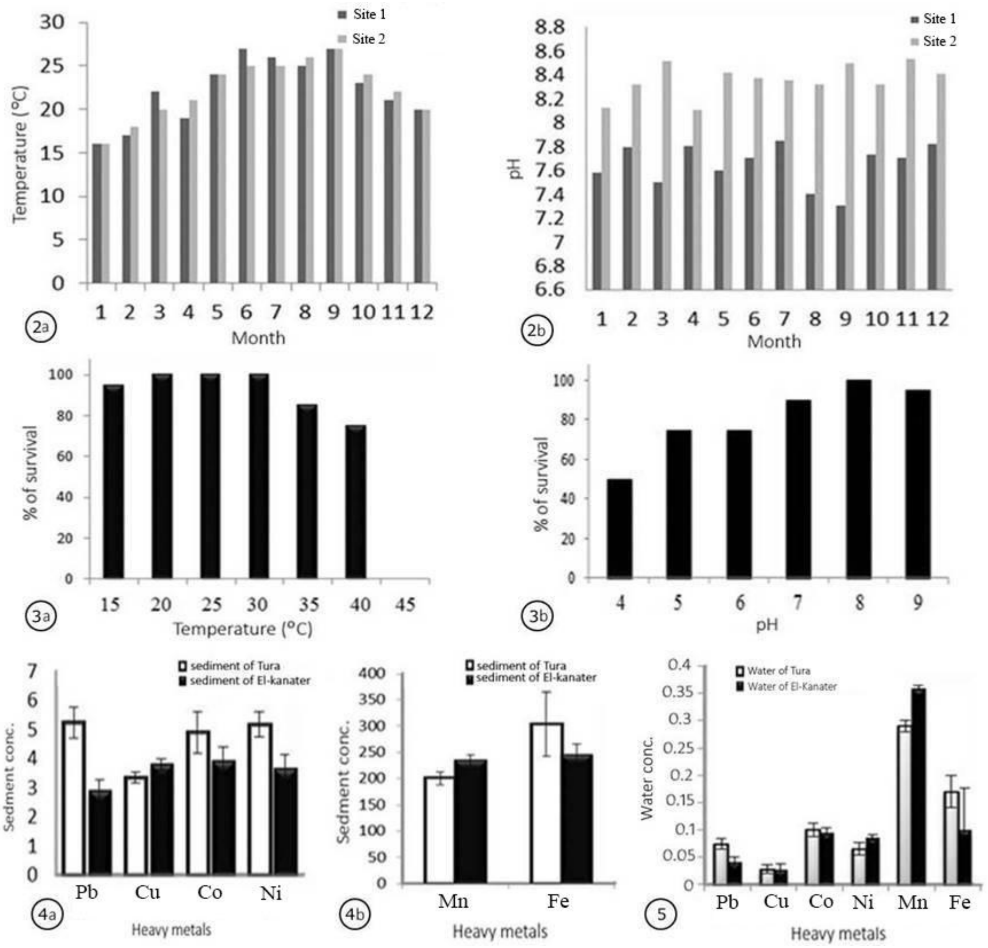
3.1.3. Effect of pH on mussel species survival showed that the ambient pH does not greatly limit the distribution of these freshwater bivalves; the majority of mussels prefer water of pH above 7.0, but in acidic medium, mussels revealed a decline in the survival.50% of the mussels survived at pH 4, 75% at pH 5-6 and 90% were alive at pH 7, while 95-100% of the studied species survived at pH 8-9 (Fig. 2b).

3.2. Water and sediment analysis (Figs. 3-5)

Mean values of trace elements measured in the water and sediment of the studied areas (El-Kanater and Tura regions), are given in Table (1). There was recorded in Cu and Fe measured in the water and sediment in both sites revealed no significant difference (P>0.05) while, there was significant difference (P<0.0001) in the concentration of Pb measured in the sediment and water of the two localities. Also, Co recorded in water showed significant difference (P<0.0001) between both sites. Ni and Mn revealed, too, (P<0.0001) in the sediment between the two studied regions. Cu, Mn and Fe measured in Nile water in both regions were in permissible levels, while, the levels of Co, Pb and Ni exceeded these levels (Table 1).

3.3. Tissue Analysis

Mean values of the measured trace elements in different soft parts (foot, mantle, gill, and digestive tissue) of *N. teretiuscula* and *S. rubens* collected from both localities under investigation are given in Table (1). There was a great fluctuation in the amount of the trace elements accumulated in the different soft parts of the studied mussels. Fe and Mn showed the highest level of metals accumulated in the different soft parts. Other metals fluctuated in their levels of accumulation. Pb showed high level of accumulation in the foot of *S. rubens* collected from Tura region. Cu showed high concentration level in the foot of *N. teretiuscula* collected from Tura region. Also, Ni showed high concentration in the mantle and gills of the studied species collected from El-Kanater region and Co recorded high accumulation level in the mantle of *S. rubens* collected from El-Kanater. Mn showed high concentration level in the mantle of *S. rubens* collected from Tura region and Fe recorded also high concentration in the digestive tissues of *S. rubens* at El-Kanater region. In general, significant difference was recorded in the concentration of the studied heavy metals in the different soft parts, between the two studied species (P<0.05, P<0.0001), at the two localities under investigation, except in some instance. Insignificant difference (P>0.05) was recorded between the mussel species as regard Co concentration in the foot of species collected from Tura as well as for the Ni, Mn and Fe concentrations in the foot of species collected from El-Kanater. Also, Cu concentration



Figs. 2-5: Histograms of different determined parameters during the period of current investigations showing: **2a** Temperature (°C) and **2b** pH of the River Nile water, at both sites under investigation (Site 1: Tura, site 2: El-Kanater). **3a** Effect of increasing Temperature (°C) and **3b** pH on the survival of the studied mussel species.**4a,b** Concentrations of heavy metals (Pb, Cu, Co and Ni, Mn, Fe) in the sediment at Tura and El-Kanater regions. **5** Concentrations of heavy metals (Pb, Cu, Co, Ni, Mn and Fe) in the water of the River Nile at Tura and El-Kanater

showed insignificant difference (P>0.05) in the mantle and gills of the two studied species from El-Kanater. Pb and Fe concentration revealed insignificant difference (P>0.05) in gills of specimens from Tura.

Table 1: Mean concentration of heavy metals (in ppm) in water, sediment (g /kg/) of Tura and El-Kanater regions and different tissues (g /kg/ dry wt) of *N. teretiscula* and *S. rubens*

Study area	Parameters \ Heavy metals		Pb	Cu	Co	Ni	Mn	Fe
Tura region	Water samples		0.05±0.01	0.022±0.008	0.22± 0.13	0.06±0.012	0.052±0.01	0.26± 0.03
	Sediments samples		5.24± 0.53	3.34± 0.2	3.9± 0.72	5.32± 0.43	198.7±12.2	303.26±60.7
	N. teretiscula	Gills	7.27 ± 1.1	5.9 ± 0.44	7.3 ± 0.76	6.67 ± 0.44	261.8 ± 25.5	347 ± 22.54
		Mantle	9.4 ± 1.1	3.99 ± 0.4	5.1 ± 0.7	2.5 ± 0.6	376.7 ± 43	511.86 ± 40
		Foot	7.93 ± 0.90	17.65 ± 3.93	6.57 ± 2.38	5.7 ± 2.95	592.4 ± 164	586.66 ± 12.6
		Digestive tissue	4.34 ± 0.2	6.65 ± 0.44	1.26 ± 0.3	5.8 ± 0.99	156.5 ± 37	669.45 ± 27.4
	S. rubens	Gills	6.33 ± 1.17	7.32 ± 0.87	9.3 ± 1.1	6.94 ± 1.29	140.75 ± 47	424.8 ± 33.67
		Mantle	2.59 ± 0.7	3.9 ± 0.34	2.57 ± 0.55	6.13 ± 1.3	649 ± 82.9	638.4 ± 54.6
		Foot	16.1 ± 4.1	11.34 ± 5.28	7.36 ± 3.2	0.94 ± 0.28	525.2 ± 13.8	672 ± 16.7
		Digestive tissue	3.3 ± 0.6	8.4 ± 0.7	1.63 ± 0.3	7.3 ± 1.5	115.4 ± 15.4	788.7 ± 22.47
El-Kanater Region	Water samples		0.032±0.008	0.03±0.01	0.09±0.007	0.05±0.005	0.04±0.004	0.14± 0.076
	Sediments samples		2.7± 0.4	3.2± 0.19	3.7± 0.47	3.64± 0.48	234.67±10.6	245.08±20.9
	N. teretiscula	Gills	2.54 ± 0.58	11.9 ± 0.98	5.45 ± 0.79	11 ± 0.47	282.0 ± 17.6	144.49 ± 24.8
		Mantle	3.3 ± 0.76	7.22 ± 1	11.69 ± 1.52	11.57 ± 3.2	475.3 ± 70.7	481.6 ± 74.27
		Foot	9.1 ± 0.5	6.4 ± 1.23	3.1 ± 0.2	3.17 ± 0.54	263 ± 37.2	339.3 ± 34.3
		Digestive tissue	7.44 ± 1.1	4.9 ± 0.47	1.1 ± 0.4	2.7 ± 1.3	358 ± 120.8	235.2 ± 93.9
	S. rubens	Gills	2.94 ± 0.62	13.45 ± 1.96	9.99 ± 1.5	11.2 ± 0.86	498.1 ± 72.8	152.47 ± 23.4
		Mantle	7.63 ± 2.13	7.15 ± 0.89	14.56 ± 2.4	11 ± 2.5	568.2 ± 105	473.8 ± 47.26
		Foot	12 ± 1.3	8 ± 0.7	5.5 ± 0.67	3.84 ± 0.7	306.1 ± 86.3	453.3 ± 200.2
		Digestive tissue	7.44 ± 1.1	6.4 ± 0.89	2.3 ± 0.54	6.1 ± 0.5	628.9 ± 53.7	911.69 ± 85
Permissible levels / water			0.01	2.0	0.001	0.02	0.4	1.0
Permissible levels / tissues			0.00025	0.003	0.002	0.0005-0.001	0.002-0.009	0.043

*Permissible levels of heavy metals in water (ppm) according to WHO (2008, 1996)
*Permissible levels of heavy metals in tissues (g/kg/ dry wt) according to FAO/WHO, (1999)
***Significant at P<0.05, P<0.0001and insignificant at P>0.05

Also, insignificant difference (P>0.05) was recorded in the concentration of Cu, in the gills of studied mussels collected from El-Kanater. Heavy metals analyzed in all tissues of the species under investigation exceeded the permissible levels according to WHO (1989) and FAO/WHO (1999). Statistically, significant difference (P<0.0001) was recorded between the different analyzed tissues as regard accumulation of heavy metals at both sites of study, for mussels under investigation. The calculated mean bioconcentration factor (BCF) of Pb, Cu, Co, Ni, Mn and Fe in the different soft parts of *N. teretiuscula, S. rubens* and the Nile water samples is given in Tables (2). The BCF of Mn revealed the highest levels among the other metals, observed in the digestive tissue of *N. teretiuscula* collected from El-Kanater followed by Fe recorded in the digestive tissue of *N. teretiuscula* and Cu in the foot of *S. rubens* from Tura. While, the highest BCF value of Pb detected in the foot of *N. teretiuscula* collected from El-Kanater. Finally, Ni found highest BCF in the digestive tissue of *N. teretiuscula* and Co recorded highest BCF in the gills of *N. teretiuscula* collected from El-Kanater. The calculated transfer factor (TF) in the different tissues from water and sediments at the two localities is shown in Tables (3, 4). Results show that the TF of the sediments was greater than that of water.

4. Discussion

The numbers of threatened bivalves and their extinctions increased at an alarming rate [83]. Molluscs are one of the most endangered taxonomic groups worldwide [48].

Table 2: Mean bioconcentration factor (BCF) of the different heavy metals in *N. teretiuscula* (A) and *S. rubens* (B) (g/kg dry weight) from Tura (I) and El-Kanater (II) regions

Heavy metals		**Pb**		**Cu**		**Co**		**Ni**		**Mn**		**Fe**	
Tissue \ Site		**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Foot	**A**	274.4	417.2	379.1	227.1	20.6	60.9	9.2	76.8	9046.2	8056.6	2776.6	3238
	B	123.5	315.2	620.5	287.1	17.6	34.4	4.6	63.4	11327.7	6920.3	3345	2423.9
Mantle	**A**	79.2	323.1	325	142.5	56	56.6	15.7	50	12088.7	4685.3	2787.2	2546.4
	B	159	155.2	328.2	140.7	45	42.2	13.4	86	10112.8	9913.2	2832.9	3656
Gill	**A**	52.9	250.7	499.1	209.3	14.5	103.3	14.7	113	3037.94	11763.2	850.5	1870.6
	B	61.3	183.8	611.4	190	21	80.9	13.8	100.7	10597.7	3177.6	622.5	1605.8
Digestive tissue	**A**	176	112.4	223.6	296.9	4.2	18	9.5	146	13381.5	1839.5	1383.3	5419.3
	B	155	118.6	289.5	234.3	8.8	14	9.3	116	7616.8	3037.4	5362.9	4781.8

Within these groups, unionids and mutelids are highly declining globally due to alteration in habitat, extinction of fish host populations, pollution and environmental changes, pointing toward future extinction [87]. They are the most imperiled group and many species became extinct in several parts of the world including Egypt, while, others are threatened or endangered [71]. Only fossils of genus *Unio* were recorded from El Fayoum, Komombo, and Idfu from Upper Egypt after their extinction from River Nile [89]. However, they proclaimed the occurrence of *Coelatura (Horusia) bourguignati* in Lower Egypt, which has other name, *Unio stagnorum*, being known only from the lower Congo Basin [27]. Thus, occurrence of *Unio* species in Egypt is doubtful.

Table 3: Mean transfer factor (TF) of the different heavy metals in the different soft parts of *N. teretiuscula* and *S. rubens* (g/kg dry wt) and in Nile water samples (mg/L) from Tura and El-Kanater regions

Site	Species	Heavy metals	Pb	Cu	Co	Ni	Mn	Fe
Tura region	*N. teretiuscula*	Water/ Gill	0.007	0.004	0.03	0.009	0.0002	0.0007
		Water/Mantle	0.005	0.0055	0.04	0.02	0.0001	0.0005
		Water/ Foot	0.006	0.001	0.03	0.01	0.0001	0.0004
		Water/Digestive tissue	0.01	0.003	0.2	0.01	0.0003	0.0004
		Sediment/ Gill	0.7	0.6	0.5	0.8	0.8	0.9
		Sediment/Mantle	0.6	0.8	0.8	2.1	0.5	0.6
		Sediment/ Foot	0.7	0.2	0.6	0.9	0.3	0.5
		Sediment/ Digestive tissue	1.2	0.5	3.1	0.9	1.3	0.5
	S. rubens	Water/ Gill	0.008	0.003	0.024	0.009	0.0004	0.0006
		Water/Mantle	0.02	0.006	0.09	0.01	0.0001	0.0004
		Water/ Foot	0.003	0.002	0.03	0.064	0.0001	0.0004
		Water/Digestive tissue	0.02	0.003	0.1	0.008	0.0005	0.0003
		Sediment/ Gill	0.8	0.5	0.4	0.8	1.4	0.7
		Sediment/Mantle	2.0	0.9	1.5	0.9	0.3	0.5
		Sediment/ Foot	0.3	0.3	0.5	5.7	0.4	0.5
		Sediment/ Digestive tissue	1.6	0.4	2.4	0.7	1.7	0.4
El-Kanater regions	*N. teretiuscula*	Water/ Gill	0.01	0.003	0.02	0.0045	0.0001	0.001
		Water/Mantle	0.01	0.004	0.008	0.0043	0.0001	0.0003
		Water/ Foot	0.004	0.005	0.03	0.02	0.0002	0.0004
		Water/Digestive tissue	0.004	0.006	0.08	0.02	0.0001	0.0006
		Sediment/ Gill	1.1	0.3	0.7	0.3	0.8	1.7
		Sediment/Mantle	0.8	0.4	0.3	0.3	0.5	0.5
		Sediment/ Foot	0.3	0.5	1.2	1.1	0.9	0.7
		Sediment/ Digestive tissue	0.4	0.7	3.4	1.3	0.7	1.0
	S. rubens	Water/ Gill	0.01	0.002	0.009	0.0045	0.0001	0.0009
		Water/Mantle	0.004	0.004	0.006	0.0045	0.0001	0.0003
		Water/ Foot	0.003	0.004	0.02	0.01	0.0001	0.0003
		Water/Digestive tissue	0.004	0.005	0.04	0.008	0.0001	0.0002
		Sediment/ Gill	0.9	0.2	0.4	0.3	0.5	1.6
		Sediment/Mantle	0.4	0.4	0.3	0.3	0.4	0.5
		Sediment/ Foot	0.2	0.4	0.7	0.9	0.8	0.5
		Sediment/ Digestive tissue	0.4	0.5	1.6	0.6	0.4	0.3

Environmental factors influence bivalve distribution, shell deposition and dissolution rates as water depth, temperature, and pH [74]. In the present study, it was found that *N. teretiuscula, S. rubens* could withstand various temperatures ranging from 15-40°C, with optimum temperatures of 25-30°C but at temperature greater than 40°C, mussels started to die and all specimens are dead at temperature above 45°C. Also, Balcom [8] found that mussels tolerate a wide range of temperature between 2 and 30°C. additionally, the effect of pH on the survival of studied mussels showed that the ambient pH does not greatly limit the distribution of these freshwater bivalves; the majority of mussels prefer water of pH above 7.0, but in acidic medium, mussels revealed a decline in their survival. This observation comes with line of Okland and Kupier [59] they stated that bivalves' diversity declines in acidic habitats. Also, McMahon and Bogan [52] revealed that uniondeans can grow and reproduce over a pH ranging from 5.6 to 8.3, but some sphaeriids are relatively insensitive to high pH (alkaline waters).

Highly acidic waters cause shell erosions and eventual death at high acidity, a pH of less than 4.7-5.0 being the absolute lower limit [32, 59] because low pH may have sublethal effecs on unionideans [29]. On the other hand, using chemical wastes and agricultural drainage systems represents the most dangerous form of the chemical pollution particularly heavy metal aquatic environment. So, contamination of natural waters by heavy metals negatively affects aquatic biota and retains considerable environmental risks [25, 67]. Therefore, water quality is considered the main factor controlling the state of health and disease in both man and animal [66]. A number of workers such as Czarnezki [18], Foster and Bater [24], Tay et al. [79] examined potential use of bivalves as biomonitoring tools especially for metals. However, it is impossible to establish accurate relationship between trace elements concentrations as bioindicators in aquatic environments and the current level of water contamination at the time of collection [68].

In the present study, analysis of six heavy metals in the soft parts of the freshwater mussels, River Nile water and sediment, collected from both regions (El-Kanater and Tura) under investigation showed that concentrations of these trace elements were lower in River Nile water than in sediment and soft parts of the studied mussels. This agreed with observation of Mackevičiene et al. [49] and Spencer and MacLeod [76] reported that sediments accumulate higher concentration of contaminants and may act as long-term stores for metals in the environment. Exposure of sediment-dwelling organisms, such as mussels, to metals may then occur via uptake of interstitial waters, ingestion of sediment particles via food chain, and threaten their life [47]. Consequently, pollutant concentrations in the organism are the results of accumulation during the past as well as the current pollution levels in the environment where organism lives but pollutant concentration in water only indicates the situation at the time of sampling [19, 31]. The concentration of heavy metals analyzed in Nile water from Tura and El-Kanater regions were not the same and found to be in the following decreasing order Mn>Fe>Co>Pb>Ni>Cu, and Mn>Fe>Co>Ni>Pb>Cu, respectively. In sediment, metals were also differently concentrated and were in sequence of Fe>Mn>Ni>Pb>Co>Cu at Tura region and Fe>Mn>Co>Cu> Ni>Pb at El-Kanater region. Fe and Mn show high concentration levels in both water and sediment samples collected from the studied area. Concentration of analyzed metals were found to be higher in sediment than water this may reflect the ability of the sediment to act as sink as suggested by Wang and Rainbow [82] particularly heavy metals may serve as an enriched source for benthic organisms. Generally, significant difference ($P<0.0001$) between heavy metal levels analyzed in water and sediment, of the two regions under investigation was recorded.

Trace elements recorded in Nile water in both regions were in the permissible levels for Mn, Cu, and Fe, while that of Ni, Co and Pb exceeded the permissible levels, which put public health and aquatic species in danger. No record of permissible levels for heavy metals in sediment is available. Therefore, Tura region belong to Helwan Governorate is considered a polluted area this agreed with observations of [21] they reported that wastewater from electroplating industry at Helwan had higher concentrations of chromium, manganese and zinc than the allowable limits. They also stated that important elements in wastewater are Hg, Pb, Cu, C, Fe, Mg, Zn and Cd. Also, Ahmed [4] reported that Helwan is the most polluted area among different studied localities along the River Nile and Ismailia canal where concentrations of Pb, Hg and Cd were 0.257, 0.12 and 0.017 mg/l, respectively. In addition, Salah et al. [73] recorded that Helwan is the most polluted area along the River Nile in Egypt, where a number of factories discharge their effluents directly to the Nile water. Fe and Mn are essential for mussel growth and reproduction as well as cobalt and copper, but at higher concentration than required, they become toxic. Pb, Cd and Ni are toxic elements and have no beneficial or desirable nutritional effects on animals [30].

Copper is one of several heavy metals that are essential to life despite being as inherently toxic as non-essential heavy metals exemplified by Lead (Pb) and Mercury (Hg) [39]. It is toxic at very low concentration in water and is known to cause brain damage in mammals [46, 49]. The toxicity of copper to freshwater mussels has been investigated by several researchers [43]. Non-essential metals like Aluminium (Al), Cadmium (Cd) and Lead (Pb) exhibit extreme toxicity even at trace levels [40]. Lead is defined by United States Environmental Protection Agency (USEPA) as potentially hazardous to most forms of life, and is considered toxic and relatively accessible to aquatic organisms [50, 57, 81]. The chronic effect of lead on man includes neurological disorders, especially in fetus and in children [55]. Cobalt has been found to have reproductive and developmental effects in animals. It is an essential metal and a component of vitamin B12 but, amount higher than required may be toxic [86]. Iron, is one of the most abundant metals, and an integral part of many proteins and enzymes that maintain good health [35]. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity [60]. On the other hand, excess amounts of iron in man can result in toxicity and even death [58]. Manganese (Mn) is one of the most abundant elements in the earth's crust and is widely distributed in soils, sediments, rocks, water, and biological materials. Sludge and various waste waters containing manganese are used in the production of micronutrient fertilizers and manganese slurries have been used in the production of clay blocks for road construction and these can serve as sources of manganese in the environment [64]. It is an essential trace element for both animals and man necessary for the formation of connective tissue and bone, and for growth, carbohydrate and lipid metabolism, the embryonic development of the inner ear, and reproductive functions [56, 65].

Table 4: Relation between heavy metals and the tissues of *N. teretiuscula* and *S. rubens* from Tura and El-Kanater regions

Fixed parameters	Heavy metals	P-value
Regions Tura *vs* El-Kanater	Lead	$P < 0.0001$
	Copper	
	Cobalt	
	Nickel	
	Manganese	
	Iron	
Species *N. teretiuscula vs S. rubens*	Lead	$P < 0.0001$
	Copper	
	Cobalt	
	Nickel	
	Manganese	
	Iron	
Tissues (Foot *vs* Mantle *vs* Gill *vs* Digestive tissues)	Lead	$P < 0.0001$
	Copper	
	Cobalt	
	Nickel	
	Manganese	
	Iron	
Regions * Species	Lead	$P < 0.0001$
	Copper	
	Cobalt	
	Nickel	
	Manganese	
	Iron	
Regions * Tissues	Lead	$P < 0.0001$
	Copper	
	Cobalt	

	Nickel	
	Manganese	
	Iron	
Species * Tissues	Lead	P < 0.0001
	Copper	
	Cobalt	
	Nickel	
	Manganese	
	Iron	

To study the possibility to use mussel species as bioindicators for pollution a question is raised and which part of the mussel (the shell or the soft parts) are able to accumulate higher metal concentration. Langston et al. [42] demonstrated that soft tissues accumulate higher metal concentrations than shells, while, others postulated that, sometimes mollusc's shells accumulate higher metal concentrations than soft tissues [78, 79]. Moreover, Bourgoin [10] and Lingard et al. [45] have earlier reported that shells have important practical advantages over soft tissues to monitoring metal contamination of aquatic environment, since shells show less variability, integrated elemental concentrations over life of animal and preserve metals after death, giving an idea about what were the concentrations in the past [15, 78]. While, Green et al. [28] and Imaly [34] have claimed that shells of bivalves are not suitable indicators of exposure history due to analytical difficulties and variation in shell metal contents associated with differences in bivalve age and growth rate. Therefore, accumulation of heavy metals was estimated here in soft parts of studied mussel species .If the degree of accumulation of any pollutant reflects the power of organism as a biomonitoring of this pollutant, so which tissue can be used as a model bioindicators for accurate accumulation all over the seasons?

Unfortunately, no definite map illustrates the tissue that can be used as the best bioindicator [20]. In the present investigation, all the tissues analyzed showed significant difference (P<0.0001) in the accumulation level of heavy metals. But, there was great variation in the amount of trace elements. Fe and Mn showed the highest level of accumulation in the soft parts of *N. teretiuscula* and *S. rubens*, while, other metals fluctuated in their level of accumulation. However, all heavy metals were accumulated at higher levels than permissible levels according to FAO/WHO [23]. These results support the study of El-Assal et al. [19] they recorded high concentration of Fe and Mn in soft parts of *Coelatura* species collected from El-Kanater and Geziret El-Daheb. Pb showed high level of accumulation in the foot of *S. rubens* collected from Tura region. Cu showed high concentration level in the foot of *N. teretiuscula* collected from Tura region. Also, Ni showed high concentration in the mantle and gills of the studied species collected from El-Kanater region and Co recorded high accumulation level in mantle of *S. rubens* collected from El-Kanater. Mn showed high concentration level in the mantle of *S. rubens* collected from Tura region and Fe recorded also high concentration in the digestive tissues of *S. rubens* at El-Kanater region. Also, Yilmaz et al. [88] in Turkey, found that it was very difficult to compare the heavy metals concentrations, even between the same tissues of two different species, because of different feeding habits, the differences in the aquatic environments concerning the type and level of water pollution, growing rates of species, types of tissues analyzed and other factors. Moreover, Kanakaraju et al. [41] stated that the accumulation metals were generally found to be scattered in different tissues of razor clam collected from Serpan with higher concentration recorded for Fe and Mn and least abundant element was Cd. Therefore, it is recommended to use the whole soft body mass to spare time and costs, using complicated and expensive techniques.

Variability of heavy metals observed in mussel species at both sites could resulted from fluctuating environment due to tidal activities, input of heavy metals from natural and anthropogenic sources, other environmental conditions such as pH and salinity and also physiological condition of studied species [41]. The elevated concentration of Fe in soft tissue of studied mussel species compared to other metals might be due to major role played by this essential metal in maintaining the proper physiological functions in the organism. This corresponds well with previous study of Kamaruzzaman et al. [40] who declared that Fe plays an important role as an essential element in all living systems from invertebrates to human; hence they tend to accumulate high concentration of Fe from environment. Furthermore, this occurrence also indicates the natural capacity of bivalve to regulate and accumulate elevated concentration of Fe [41]. Accordingly, *N. teretiuscula* and *S. rubens* can be used as bioindicators for heavy metals pollution in freshwater ecosystems. This result agreed with data obtained from other studies of Burgos and Rainbow [13] and Moloukhia and Sleem [53] on some freshwater bivalves, also, they observed that heavy metals were accumulated mainly in soft parts, while small amounts were absorbed by their shells. Studied mussel show the same ability to accumulate heavy metals. But, none of mussel soft tissues have advantage over the other, and the whole soft parts can be used in this respect.

The soft tissues can effectively reflect the environmental bioavailability of these metals better than water and sediment. Bioconcentration factor (BCF) represents the degree of accumulation of metal in organism relative to its environment. The present results showed bioconcentration factor (BCF) of Mn and Fe revealed high levels in both sites and in different tissues of studied mussels, followed by Cu and Pb, while, BCF of Ni and Co recorded the lowest level. Data of BCF reported here confirms the ability of studied species to accumulate heavy metals in high concentration and provide a useful approach to biological monitoring of past and current exposure to essential and non-essential elements. Similarly, El-Gamal and Sharshar [20] recorded the BCF values of heavy metals (Fe, Pb, Cd, Co, Cu and Zn) accumulated in the soft parts of *Coelatura nilotica*, and found that the BCF of all the studied metals was high (except for Cu and Pb), indicating the ability of this species to accumulate heavy metals. Moreover, the present results showed that the transfer factor (TF) of all elements in mussel tissues from the sediments was greater than that from water, as the metal concentrations are higher in sediment. This confirmed again the ability of sediment to act as sink as claimed by Olowu et al. [62]. No record was reported previously, for transfer factor in the studied species tissues from sediments and Nile water and it is the first time to be reported here. Finally, *N. teretiuscula* and *S. rubens* can be used as bioindicator of metal pollution in freshwater ecosystems.

In fact, aquatic bivalves in Egypt are endangered due to heavy metal pollution of the Nile River. .Also, these heavy metals accumulated within the mussels tissues and can pass to human through the food chain. Thus water quality is considered the main factor controlling the state of health and disease in both man and animal. Generally, heavy metals analysis is important in two main aspects, the public health point of view and the aquatic environment conservation. Therefore, studies of chemical quality of aquatic organisms, particularly the concentrations of heavy metals are extremely important.

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6. Conflict of interest

Authors have indicated that they have no conflict of interest regarding the content of this article.

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