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Pollution of Freshwater *Coelatura* species (*Mollusca: Bivalvia: Unionidae*) with Heavy Metals and its impact on the Ecosystem of the River Nile in Egypt

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Abstract

The Knowledge of heavy metal concentrations in aquatic species is important with respect to genetic variation and extinction of some species and loss of biodiversity in the ecosystem of rivers and lakes. We used random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) to examine genetic differentiation among *Coelatura* species collected from the River Nile, at two polluted locations (El-Kanater, Qalyoubyia governorate and Tura, Cairo governorate, Egypt) and the impact of heavy metal pollution on the genetic structure of these species (*C. aegyptiaca, C. prasidens, C. canopicus, C. gaillardoti* and *C. parreyssi*). RAPD PCR was carried out using five random primers (UBC 476, UBC 477, UBC 478, UBC 479 and UBC 487) that provided strong amplifications. The RAPD- PCR analysis between any given pair of species, based on the number of bands, showed natural differences or polymorphism among the *Coelatura* species under investigation. The greatest number of PCR fragments was found with primers UBC 478 and UBC 479 (6-7 bands), while less fragments were obtained with primers UBC 476, UBC 477 and UBC 487 (2-4 bands)

Primers UBC 477 and UBC 479 clearly distinguished the five studied *Coelatura* species into only three species, *C. aegyptiaca, C. parreyssi* and *C. canopicus* and primer UBC 478 showed DNA alteration concerning *C. parreyssi*, *C. gaillardoti* and *C. canopicus.*

Genetic diversity was also measured as the percentage of polymorphic bands for each primer.

The dendograms and the similarity index (D) showed, also, that the five studied species could be classified into only three species, *C. aegyptiaca, C. canopicus* and *C. parreyssi*

The concentration of six heavy metals (copper, cobalt, nickel, manganese, lead and iron) was determined in the soft parts of the *Coelatura* species to assess the impact of heavy metal pollution on their genetic variation. Metal concentrations in the tissues were found to be higher than the permissible limits, indicating that heavy metals might play an important role in the genetic variation of *Coelatura* species by inducing DNA damage and alteration of the genetic pattern as well as they may be the cause of the extinction of some species and the loss of biodiversity in the ecosystem of the freshwater ecosystem.

Keywords: *Coelatura*; *C. aegyptiac;, C. prasidens; C. canopicus; C. gaillardoti; C. parreyssi;* Taxonomy, RAPD-PC; Genetic variation; Heavy metals; Pollution

Introduction

Pollution of the freshwater environments by heavy metals due to increased action of flowing out discharge from various industries has received considerable attention in that it is able to influence freshwater organisms, leading to modify their genetic diversity [1-7]. Pollution affects adversely organisms and could be the cause of the genetic variation of some species.

Metal exposure was found to lead to various types of DNA damages and alteration of genetic patterns within populations [8,9] and also, DNA damage may indicate levels of metal toxicity.

In Egypt, *Coelatura* species showed great argument on their taxonomy, and their number ranged from 1 to 14 species in various studies [10-12] which consequently lead to questionable taxonomy. Therefore, in the present study, we used RAPD-PCR method to resolve the conflict on the taxonomical status of some *Coelatura* species from the River Nile in Egypt and to discuss the effect of metal pollution in this respect.

On the other hand, *Unionidae* are declining at a catastrophic rate worldwide. They are threatened by a number of factors among which industrial and human activities inducing environmental pollution [13], pointing toward impending mass extinction. The significant loss of biodiversity may permanently alter ecosystem functioning in rivers and lakes as well as alter the rate of ecological processes [14].

Metal pollution of freshwater sources appears to be the main cause of the endangerment of freshwater mussels which are endangered nowadays worldwide and it is possible that high amounts of metals are toxic and could be a contributing threatening factor [15]. Therefore, it is important to estimate the accurate levels of trace elements in some mussels' species (*Coelatura* species as example) to assess the impact of heavy metals on their genetic variation and on the loss of biodiversity in the ecosystem of the River Nile in Egypt.

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Material and Methods

Collections of samples

The *Coelatura* species *(C. aegyptiaca, C. prasidens, C. gaillardoti, C. canopicus and C. parreyssi)* were collected from the River Nile at two localities, known to be heavy metal polluted (El-Kanater, Qalyoubia Governorate and Tura, Cairo Governorate). Samples were monthly and randomly collected, for one year, from September 2009 to August 2010, then transported to the laboratory, sorted and maintained under the same conditions of food and temperature

Genetic study

DNA extraction and RAPD-PCR analysis: Samples of the *Coelatura* species were dissected and their soft parts were preserved in 100% ethyl alcohol at -20°C until used. Total genomic DNA was extracted from frozen ethanol-preserved mantle using Qiagen Dneasy tissue kit (Valencia, CA, USA) according to the manufacturer's manual. Seven primers were used in the present work, which were previously used in the bivalve RAPD-PCR [11,16,17].

476: 5` - TTG AGG CCC T – 3`

477: 5` -TGT TGT GCC C – 3`

478: 5` - CGA GCT GGT C – 3`

479: 5` - CTC ATA CGC G – 3`

483: 5` - GCA CTA AGA C– 3`

486: 5` - CCA GCA TCA G – 3`

487: 5` - GTG GCT AGG T – 3`

Only five primers worked out (UBC 476, UBC 477, UBC 478, UBC 479 and UBC 487). Amplifications were performed by modifying the protocol reported by Williams et al (1990) [18]. The 25 µl mixture contained 25 ng of template DNA, 1.5 unit of Taq Polymerase, 10 mMdNTPs, 10 pM primer, and 2.5 µl of 10x PCR buffer. (Dream Taq Green PCR MasterMix (2X) (Fermentas). Each amplification reaction was performed using a single primer and repeated twice to verify band autosimilarity [19].

Amplifications were performed in T-personal thermal cycler (Techne, TC-3000G), programmed for 45 cycles of 94°C for 1 minutes., 35°C for 1 minute., and 72°C for 1 minute. An initial denaturation step (3 minutes, 94°C) and a final extension holding (10 minutes, 72°C) were included in the first and last cycles, respectively.

Ten µl of the reaction products were resolved by 2% agarose gel electrophoresis at 85 volt in 1x TAE (Tris-acetate-EDTA) buffer. The gel was stained with ethidium bromide and photographed by a digital camera under UV transilluminator. For the comparison of the amplified products, population-specific fragments were detected using Gene Ruler 1 kb Plus DNA Ladder from Fermentas.

Molecular data analysis: Molecular data analysis was carried out using gel documentation system (SynGene, GeneTools - File version: 4.02.03, France), for the dendogram and calculation of similarity index of each primer between the studied *Coelatura* species. RAPD amplification products were scored as 0/1 for absence / presence of homologous bands [20] and analyses carried out using the NTSYS PC2.0 software [21].

Similarity coefficient matrix was calculated using Jaccard similarity algorithm [22] for RAPD markers. Dendograms were constructed using the UPGMA method, Unweighed pair-Group Method with arithmetical algorithms Averages [23]. Genetic diversity was also measured as the percentage of polymorphic bands. The percentage of polymorphic RAPD loci was calculated for each species, as well as the mean and overall value for all species and each primer.

Heavy metal analysis

Water and sediment analysis: Water and sediment samples collected from the two studied regions were analysed to determine the concentrations of heavy metals, using atomic absorption spectrophotometer model A-Analyst 100 Perkin Elmer. Metals analysed were Copper (Cu), Cobalt (Co), Nickel (Ni), Manganese (Mn), Lead (Pb), and Iron (Fe).

Tissue analysis [24]: Mussels were dissected and the soft parts were excised on clean tared pieces of plastic. Wet weights were then determined by the method of Johanson et al. [25]. Tissues were dried to constant weight, at room temperature, for 24 hours, removed from the plastic pieces and placed in 1.5 ml washed micro centrifuge tubes.

To each tube, 5 ml of piperidine (mole/litre) was added, the tubes were then cooled to room temperature, after which 2 ml of 61% (V/V) $HCLO_4$ was added to the precipitate. After 10 minutes, 7 ml of deionized water was added and mixed. Fifteen minutes later, the tubes were centrifuged for one minute, at 10,000 r.p.m. in a microcentrifuge (Beck Man/ Model J-2, 21). Supernatants were added in aliquots for analysis; using an atomic absorption spectrophotometer, model A-Analyst 100 Perkin Elmer instrumentation laboratories.

Single cuvette attached to an aspiration pump was used to minimize handling of samples and absorption of each ion was integrated for 2 seconds. Metals measured in tissues were Cu, Co, Ni, Mn, Pb and Fe.

The transfer factor (TF) in mussel tissues from the aquatic ecosystem, which include water and sediments, was calculated according to Kalfakakour and Akrida-Demertzi [26] and Rashed [27] as follows:

TF= Mtissue/ Msediment or Mwater

Mtissue = the metal concentration in mussel tissue, Msediment = the metal concentration in sediment and Mwater = the metal concentration in water.

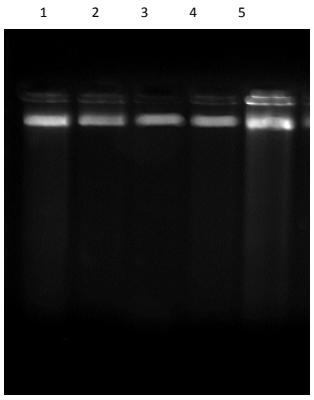

Figure 1: Agrose gel of extracted DNA from the mantle of the five *Coelatura* species under investigation.
1. *C. parreyssi*
2. *C. aegyptiaca*
3. *C. gaillardoti*
4. *C. canopicus*
5. *C. prasidens*

Statistical analysis

A software computer program SPSS Version 19 was used to test the significance differences between mean values of the different parameters in the studied mussels. One - way ANOVA and MANOVA were employed to find the difference in the ecological analysis at a probability level $P>0.05$ for insignificant results and $P<0.05$ and $P<0.0001$ for significant results.

Results

Genetic studies

Individual amplifications of agarose gel extracted DNA from the mantle of the five studied *Coelatura* species (Figure 1) were performed using the five primers UBC 476, UBC 477, UBC 478, UBC 479 and UBC 487, in order to determine the genetic relationship between them.

RAPD PCR carried out using the five primers provided strongly amplified fragments (Figures 2-6).

Genetic variability was observed among the studied *Coelatura* species. The greatest number of PCR fragments was found with primers UBC 478 and UBC 479 (6-7 bands), while less fragments were obtained with primers UBC 476, UBC 477 and UBC 487 (2-4 bands). The RAPD- PCR analysis was based on the number of bands that were different between any given pair of species (Table 1). Analyses showed natural differences (polymorphism) among *Coelatura* species under investigation.

Primers UBC 477 (Figure 3a) and UBC 479 (Figure 5a) gave monomorphic bands with *C. Parreyssi* and *C. gaillardoti* and as well as with *C. aegyptiaca* and *C. prasidens*. While, *C. canopicus* revealed some polymorphic bands (Figures 3a and 5a). Primer UBC 476 gave similar results for *C. aegyptiaca* and *C. prasidens* as with primers UBC 477 and UBC 479, but it showed monomorphic bands for *C. parreyssi, C. gaillardoti* together with *C. canopicus* (Figure 2a).

However, Primer UBC 478 (Figure 4a) showed DNA alteration concerning *C. parreyssi*, *C. gaillardoti* and *C. canopicus*. This DNA alteration might have resulted from mutation or rearrangements at or between oligonucleotide primer binding sites in a genome. Primer UBC 487 (Figure 6a) revealed monomorphic bands for all five studied *Coelatura* species.

Genetic diversity was also measured as the percentage of polymorphic bands for each primer (Tables 2 and 3). 9.26 % of the bands were polymorphic among the five studied primers. Except primer UBC 487 which revealed no polymorphism, the other primers produced 1 to 6 polymorphic bands. Some RAPD fragments were found to be unique; 1 in *C. parreyssi* and *C. gaillardoti* and 3 in *C. canopicus* (Table 3).

Considering the similarity index (D) of the *Coelatura* species (Tables 5-9), utilizing RAPD-PCR markers, species were considered

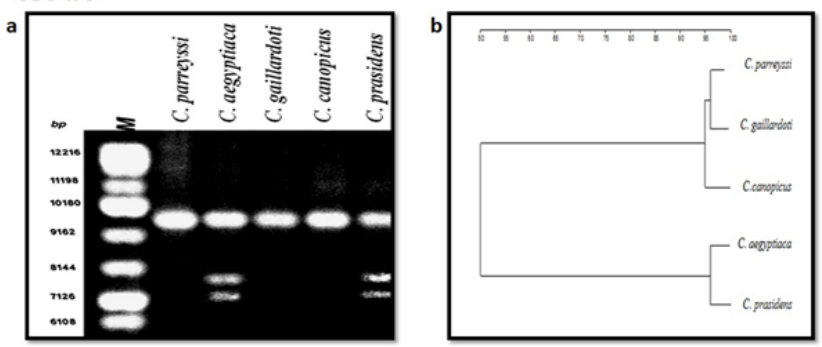

Figure 2: (a) RAPD-PCR profiles of the five *Coelatura* species under investigation using primer (UBC 476), and M: 1kb DNA marker shows one monomorphic band for all studied species and revealed 2, other monomorphic band for *C. aegyptica* and *C. prasidens*.
(b) Dendrogram of primer UBC 476 demonstrating the relationships of the five *Coelatura* species under investigation, based on compiled data set shows that *C. parreyssi, C. gaillardoti* identical species and *C. canopicus* are closed one also, *C. aegyptiaca* and *C. prasidens* similar species.

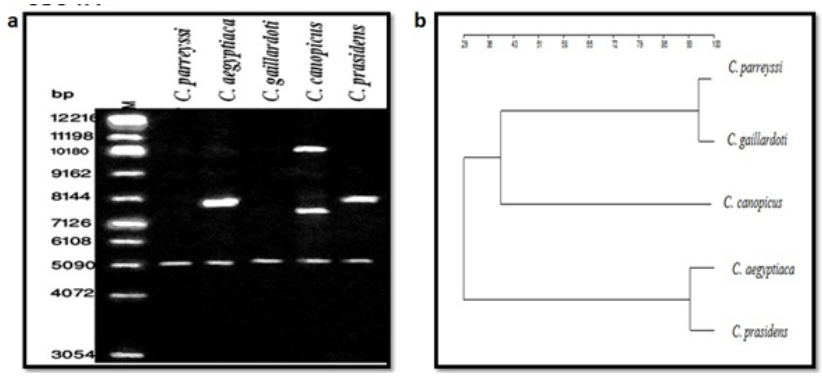

Figure 3: RAPD-PCR analysis of the five *Coelatura* species under investigation using primer (UBC 477)
(a) Gel electrophoresis showing amplification profile of samples. M: 1kb DNA marker.
(b) Dendrogram demonstrating the similarity relationship between the five *Coelatura* species under investigation.

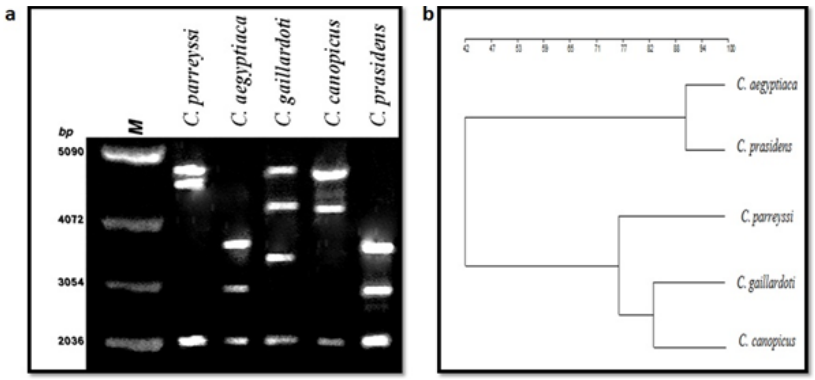

Figure 4: RAPD-PCR analysis of the five *Coelatura* species under investigation using primer (UBC 478)
(a) Gel electrophoresis showing amplification profile of samples. M: 1kb DNA marker.
(b) Dendrogram demonstrating the similarity relationship between the five *Coelatura* species under investigation.

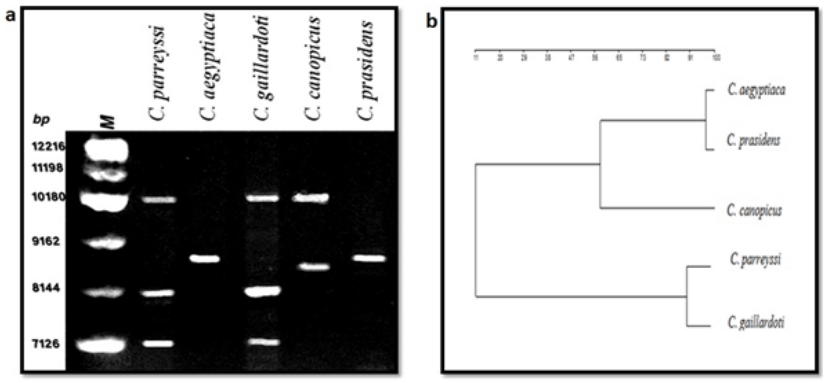

Figure 5: RAPD-PCR analysis of the five *Coelatura* species under investigation using primer (UBC 479)
(a) Gel electrophoresis showing amplification profile of samples. M: 1kb DNA marker.
(b) Dendrogram demonstrating the similarity relationship between the five *Coelatura* species under investigation.

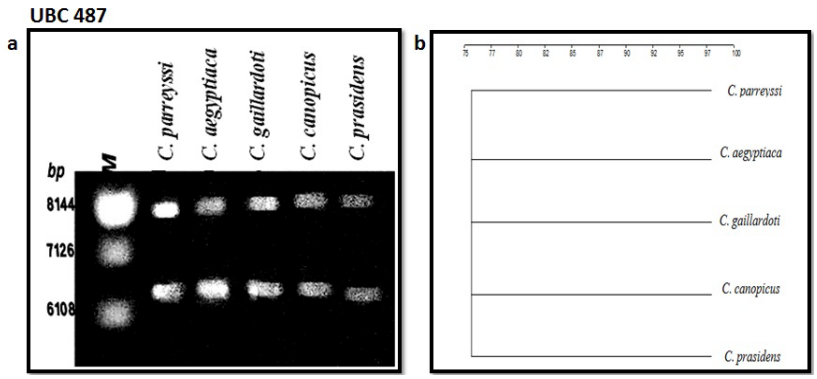

Figure 6: RAPD-PCR analysis of the five *Coelatura* species under investigation using primer (UBC 487)
(a) Gel electrophoresis amplification profile of samples M: 1kb DNA marker.
(b) Dendrogram demonstrating the similarity relationship between the five *Coelatura* species under investigation.

similar when the (D) value between two species is equal or close to 1. While, when (D) is distant from 1, the two species were regarded as separate species.

The similarity index (D) between *C. aegyptiaca* and *C. prasidens*, using all studied primers, was close to 1 (0.90-0.97), thus they were the closest species, and were considered one species, *C. aegyptiaca*. While, it was distant from 1 between these two species and the other studied species, except for primer UBC 487 which showed no polymorphism. Also, the (D) value, using the primers UBC 476 and UBC 478, was close to 1 between *C. parreyssi*, *C. gaillardoti* and *C. canopicus*. However, using

Primers/Species	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*	*C. prasidens*
UBC 476 (Figure 1a)					
1	Band 1 at ~ 9671.16 bp	Band 1 at ~ 9677.96 bp	Band 1 at ~ 9732.50bp	Band 1 at ~ 9739.76 pb	Band 1 at ~ 9759.88bp
2		Band 2 at ~ 7766.91 bp			Band 2 at ~ 7830.31 bp
3		Band 3 at ~ 7261.65 bp			Band 3 at ~ 7302.69 bp
UBC 477 (Figure 2a)					
1	Band 1 at ~ 5157.41bp	Band 1 at ~ 8004.24 bp	Band 1 at ~ 5265.15bp	Band 1 at ~ 10284.48 pb	Band 1 at ~ 8133.93 bp
2		Band 2 at ~ 5167.11 bp		Band 2 at ~ 7655.77bp	Band 2 at ~ 5265.15 bp
3				Band 3 at ~ 5265.15 bp	
UBC 478 (Figure 3a)					
1	Band 1 at ~ 4939.03bp	Band 1 at ~ 3788.28 bp	Band 1 at-4952.17 bp	Band 1 at ~ 4890.24bp	Band 1 at ~ 3716.02 bp
2	Band 2 at ~ 4737.70 bp	Band 2 at ~ 3094.70 bp	Band 2 at ~ 4398.09 bp	Band 2 at ~ 4359.32bp	Band 2 at ~ 3061.36 bp
3	Band 3 at ~ 2154.60 bp	Band 3 at ~ 2158.54 bp	Band 3 at ~ 3554.15 bp	Band 3 at ~ 2131.12bp	Band 3 at ~ 2158.54 bp
4			Band 3 at ~ 2158.54 bp		
UBC 479 (Figure 4a)					
1	Band 1 at ~ 10195.41bp	Band 1 at ~ 8834.00 bp	Band 1 at ~ 10272.82 bp	Band 1 at ~ 10319.55bp	Band 1 at ~ 8825.74 bp
2	Band 3 at ~ 8112.54 bp		Band 2 at ~ 8174.51 bp	Band 2 at ~ 8654.18bp	
3	Band 4 at ~ 7153.63 bp		Band 3 at ~ 7188.33 bp		
UBC 487 (Figure 5a)					
1	Band 1 at ~ 8093.80 bp	Band 1 at ~ 8093.80 bp	Band 1 at ~ 8193.97 bp	Band 1 at ~ 8106.52 bp	Band 1 at ~ 8156.88 bp
2	Band 2 at ~ 6542.64 bp	Band 2 at ~ 6542.64 bp	Band 2 at ~ 6556.28 bp	Band 2 at ~ 6583.66 bp	Band 2 at ~ 6529.02 bp

Table 1: Bands Pattern in *C. parreyssi, C. aegyptiaca, C. gaillardoti, C. canopicus* and *C. prasidens* using the five primers.

Primer	Total number of bands	Monomorphic	Polymorphic	% of polymorphism
UBC 476	3	1	2	66.7
UBC 477	4	1	3	75
UBC 478	7	1	6	85.7
UBC 479	5	0	5	100
UBC 487	2	2	0	0

Table 2: Total number of bands (monomorphic, polymorphic and percentage of polymorphism) of each primer, in *Coelatura* species under investigation.
*The repeated bands in all species are counted once.

Bands	Total	*C. prasidens*	*C. canopicus*	*C. gaillardoti*	*C. aegyptiaca*	*C. parreyssi*
Amplified	54	11	11	11	11	10
Monomorphic	49	11	8	10	11	**9**
Polymorphic	5	0	3	1	0	1
Unique	5	0	3	**1**	0	1
% of polymorphism	9.26	0 %	27.3 %	9.1 %	0 %	10 %

Table 3: Total number of bands for all studied primers (monomorphic, polymorphic, unique) and percentage of polymorphism, revealed by RAPD markers among the five studied *Coelatura* species.

Species	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*
C. aegyptiaca	0.53			
C. gaillardoti	0.96	0.51		
C. canopicus	0.94	0.52	0.96	
C. prasidens	0.49	0.96	0.49	0.47

Table 4: Similarity index (D) of the Egyptian *Coelatura* specie susing UBC 476 primer.

Species	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*
C. aegyptiaca	0.29			
C. gaillardoti	0.96	0.28		
C. canopicus	0.39	0.30	0.40	
C. prasidens	0.39	0.94	0.38	0.11

Table 5: Similarity index (D) of the Egyptian *Coelatura* specie susing UBC 477 primer.

the primers UBC 477 and UBC 479, (D) was distant from 1. *C. parreyssi* and *C. gaillardoti* were the most closely associated species and may be considered one species, *C. parreyssi*. While, *C. canopicus* was somewhat distant and may be regarded as distinct species or subspecies.

The dendrogram analyses, using primers UBC 476, UBC 477 and UBC 479 (Figures 2b, 3b and 5b) confirmed the results obtained with the RAPD profiles and those of the (D) value. *C. aegyptiaca* and *C. prasidens* were the closest species, as well as are *C. parreyssi* and *C. gaillardoti*. While, *C. canopicus* was a separate species. The dendogram using primer UBC 478 showed the same result for *C. aegyptiaca* and *C.*

Species	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*
C. aegyptiaca	0.55			
C. gaillardoti	0.78	0.43		
C. canopicus	0.73	0.35	0.83	
C. prasidens	0.47	0.91	0.39	0.30

Table 6: Similarity index (D) of the Egyptian *Coelatura* specie susing UBC 478 primer.

Species ar	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*
C. aegyptiaca	0.02			
C. gaillardoti	0.90	0.00		
C. canopicus	0.35	0.58	0.30	
C. prasidens	0.01	0.97	0.008	0.58

Table 7: Similarity index (D) of the Egyptian *Coelatura* specie susing UBC 479 primer.

Species	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*
C. aegyptiaca	0.91			
C. gaillardoti	0.91	0.92		
C. canopicus	0.84	0.85	0.92	
C. prasidens	0.74	0.90	0.77	0.85

Table 8: Similarity index (D) of the Egyptian *Coelatura* specie susing UBC 487 primer.
^ All studied species show high similarity index (D), ranging from 0.74 to 0.92.

Species	*C. parreyssi*	*C. aegyptiaca*	*C. gaillardoti*	*C. canopicus*
C. aegyptiaca	0.91			
C. gaillardoti	0.91	0.92		
C. canopicus	0.84	0.85	0.92	
C. prasidens	0.74	0.90	0.77	0.85

Table 9: Similarity coefficient matrix of all primers calculated by NTSYS of the Egyptian *Coelatura* species.

prasidens, while some difference was revealed concerning *C. parreyssi, C. gaillardoti* and *C. canopicus*. The two latter species were the most related species and *C. parreyssi* was somewhat distant (Figure 4b).

According to the Similarity coefficient matrix of all primers (Table 9), the highest (D) value (0.55 and 0.67) was between *C. gaillardoti* and *C. parreyssi* and between *C. prasidens* and *C. aegyptiaca*. While, the lowest D-value (0.12) was recorded between *C. gaillardoti* and *C. aegyptiaca*, *C. canopicus* and *C. aegyptiaca* and between *C. prasidens* and *C. parreyssi*. This confirms that *C. aegyptiaca* and *C. prasidens* are similar and *C. gaillardoti* and *C. parreyssi* are also similar, while *C. canopicus* is different.

Heavy metal analysis

Nile water and sediment analysis: The mean values of the concentrations of the trace elements measured in the water and sediment of the studied areas (El-Kanater and Tura regions), are given in Table 10.

There was no significant difference (P>0.05) recorded in Cu and Fe measured in the water or sediment between both sites, while, significant difference (P<0.0001) in the concentration of Pb in the sediment and water between the two localities, was recorded. Also, Co concentration in the water showed significant difference (P< 0.0001) between both sites. Ni and Mn revealed, too, significant difference (P<0.0001) in the sediment between the two regions.

Trace elements recorded in the Nile water of both regions were in the permissible levels for Cu, Mn and Fe, while the levels of Co, Pb and Ni exceeded these levels (Table 10).

The concentrations of the different studied heavy metals in water of the two locations were in the following decreasing order:

Tura region: Fe>Co>Ni >Mn>Pb> Cu

El-Kanater region: Fe>Co>Ni >Mn>Pb> Cu

Metal concentrations in the sediment of the two locations were in the following sequence:

Tura region : Fe>Mn>Ni >Pb>Co>Cu

El-Kanater region: Fe>Mn> Co>Ni>Cu>Pb

Tissue analysis

There was a great variation in the amount of the trace elements accumulated in the different soft parts of the studied mussels (Tables 11-14).

In general, significant difference was recorded in the concentration of the studied heavy metals in the different soft parts, between the studied *Coelatura* species (P<0.05, P<0.0001), at the two localities under investigation, except in some instance.

Heavy metals analyzed in all tissues of the three studied *Coelatura* species exceeded the permissible levels according to WHO [28] and FAO/WHO [29]. The calculated transfer factor (TF) in the different tissues from water and sediments at the two localities is shown in Tables 15-17. Results show that the TF of the sediments was greater than that of water.

Discussion

Human exploitation of world mineral resources and advances in industrialization has resulted in high levels of heavy metals in the environment [30-32]. The aquatic bodies near the industrial and urban areas are more able to accumulate such metals, causing hazardous impact on the freshwater fauna. The impact of metals on different bivalve populations revealed that those inhabiting environments contaminated by heavy metals exhibited a higher allelic diversity [33]. DNA damage and genetic diversity in aquatic animal populations induced by chemical contaminants have been successfully detected using RAPD method [34-37].

RAPD-PCR analysis proved to be helpful in estimating genetic variations among species [11,38]. Analyses of the RAPD-PCR showed natural differences or polymorphism among the *Coelatura* species under investigation, and distinguished them to only three species namely, *C. aegyptiaca, C. parreyssi* and *C. canopicus* which were also confirmed by dendograms and (D) values. In fact, thorough revision of genus *Coelatura* was needed by applying molecular techniques to reveal the current concept that it represents a lumped species complex, as claimed by Ortmann [39], Graf [40] and Graf and Cummings [12].

The present study shows that *C. aegyptiaca* and *C. prasidens* are closely related and could be considered as one species, *C. aegyptiaca*, which is the type species of the genus *Coelatura*. Also, *C. parreyssi* and *C. gaillardoti* are closely related and are considered as the same species, *C. parreyssi*, which has advantage over *C. gaillardoti* because of nomenclature priority [41]. On the other hand, *C. canopicus* is somewhat distant from the other studied species and may be considered a separate species or a subspecies. Finally, the similarity coefficient matrix and the UPGMA dendogram of all primers confirmed that the five *Coelatura* species under investigation should be classified into three species only namely, *C. aegyptiaca*, *C. parreyssi* and *C. canopicus* (Figure 7). Thus, assessing the genetic diversity of populations could

Metals / Parameters	Fe	Mn	Ni	Co	Cu	Pb
Sediment at Tura	303.26 ± 60.7	198.7 ± 12.2	5.32 ± 0.43	3.9 ± 0.72	3.34 ± 0.2	5.24 ± 0.53
Sediment at El-Kanater	245.08 ± 20.87	234.67 ± 10.6	3.64 ± 0.48	3.7 ± 0.47	3.2 ± 0.19	2.7 ± 0.4
P value*	P > 0.05	*P < 0.0001	*P < 0.0001	P > 0.05	P > 0.05	*P < 0.0001
Water at Tura	0.26 ± 0.03	0.052 ± 0.01	0.06 ± 0.012	0.22 ± 0.13	0.022 ± 0.008	0.05 ± 0.01
Water at El-Kanater	0.14 ± 0.076	0.04 ± 0.004	0.05 ± 0.005	0.09 ± 0.007	0.03 ± 0.01	0.032 ± 0.008
P value*	P > 0.05	P > 0.05	P > 0.05	*P < 0.0001	P > 0.05	*P < 0.0001
Permissible levels of water	1	0.4	0.02	0.001-0.002	2	0.01

Table10: Concentration of heavy metals (in ppm) in water and sediment of the River Nile at El-Kanater and Tura regions, and the permissible levels in water according to the WHO (2008, 1996).
*Significant at P< 0.0001 and insignificant at P <0.05

Heavy metals	Tura region			P- value	El-Kanater region			P- value	*Permissible levels in mg/kg
	C. aegyptiaca	*C.canopicus*	*C. parreyssi*		*C. aegyptiaca*	*C.canopicus*	*C. parreyssi*		
Lead	7.3 ± 0.80	5.93 ± 0.78	13.1 ± 3.1	***P < 0.0001	3.73 ± 0.24	0.25 (0.00025 g)	***P < 0.0001	12 ± 1.3	9.1 ± 0.5
Copper	13 ± 1.7	13.65 ± 2.93	8.34 ± 2.28	***P < 0.0001	9.35 ± 0.83	3 (0.003 g)	***P < 0.0001	6.4 ± 1.23	8 ± 0.7
Cobalt	6 ± 1	4.57 ± 2.18	5.36 ± 2.2	P > 0.05	0.83 ± 0.1	-	***P < 0.0001	5.5 ± 0.67	3.1 ± 0.2
Nickel**	6.79 ± 1.26	4 ± 2.58	0.54 ± 0.18	***P < 0.0001	3.64 ± 0.52	0.5-1.0 (0.0005-0.001 g)	P > 0.05	3.84 ± 0.7	3.17 ± 0.54
Manganese	164.4 ± 17.9	532.39 ± 136.56	425.2 ± 12.79	***P < 0.0001	223.8 ± 24.2	2-9 (0.002-0.009 g)	P > 0.05	306.1 ± 86.3	263 ± 37.2
Iron	654 ± 85.4	568.66 ± 12	472 ± 15.7	***P < 0.05	278.2 ± 24	43 (0.043 g)	P > 0.05	453.3 ± 200.2	339.3 ± 34.3

Table 11: Mean concentrations of the heavy metals in the foot of *C. aegyptiaca, C. parreyssi* and *C. canopicusin* g /kg at Tura and El-Kanater regions ± standard deviation.
*Permissible levels of heavy metals according to FAO/WHO, (1999).
**Permissible levels of Ni according to WHO (1989).
***Significant at P < 0.05, P < 0.0001and insignificant at P > 0.05

Heavy metals	Tura region			P- value	El-Kanater region			P- value	*Permissible levels in mg/kg
	C. aegyptiaca	*C.canopicus*	*C. parreyssi*		*C. aegyptiaca*	*C.canopicus*	*C. parreyssi*		
Lead	0.25 (0.00025 g)	***P < 0.0001	9.4 ± 1.1	4.5 ± 1	2.59 ± 0.7	***P < 0.0001	7.63 ± 2.13	3.3 ± 0.76	3.8 ± 0.74
Copper	3 (0.003 g)	P > 0.05	3.99 ± 0.4	3.94 ± 0.3	3.9 ± 0.34	***P < 0.0001	7.15 ± 0.89	7.22 ± 1	10.4 ±1.24
Cobalt	-	***P < 0.0001	5.1 ± 0.7	3.8 ± 0.83	2.57 ± 0.55	***P < 0.0001	14.56 ± 2.4	11.69 ± 1.52	7 ± 1
Nickel**	0.5-1.0 (0.0005-0.001 g)	***P < 0.0001	2.5 ± 0.6	4.3 ± 0.4	6.13 ± 1.3	***P < 0.05	7.9 ± 0.69	11 ± 2.5	11.57 ± 3.2
Manganese	2-9 (0.002-0.009 g)	***P < 0.0001	178 ± 58.4	376.7 ± 43	649 ± 82.9	***P < 0.0001	568.2 ± 104.7	475.3 ± 70.7	392.94 ± 19.8
Iron	43 (0.043 g)	***P < 0.0001	356.5 ± 36.2	511.86 ± 40	638.4 ± 54.6	***P < 0.0001	473.8 ± 47.26	481.6 ± 74.27	238.8 ± 31.7

Table 12: Mean concentrations of the heavy metals in the mantle of *C. aegyptiaca, C. parreyssi* and *C. canopicusin* g /kg at Tura and El-Kanater regions ± standard deviation.
*Permissible levels of heavy metals according to FAO/WHO, (1999).
**Permissible levels of Ni according to WHO (1989).
***Significant at P < 0.05, P < 0.0001and insignificant at P > 0.05.

Heavy metals	Tura region			P- value	El-Kanater region			P- value	*Permissible levels in mg/kg
	C. aegyptiaca	*C.canopicus*	*C. parreyssi*		*C. aegyptiaca*	*C.canopicus*	*C. parreyssi*		
Lead	0.25 (0.00025 g)	***P < 0.0001	7.27 ± 1.1	5.33 ± 1.1	4.85 ± 0.56	P > 0.05	2.54 ± 0.58	2.94 ± 0.62	2.35 ± 0.78
Copper	3 (0.003 g)	P > 0.05	5.9 ± 0.44	5.32 ± 0.7	5.6 ± 1.2	***P < 0.05	10.98 ± 1	13.45 ± 1.96	11.9 ± 0.98
Cobalt	-	***P < 0.0001	9.3 ± 1.1	7.3 ± 0.76	8.5 ± 1	***P < 0.0001	3.77 ± 1.2	5.45 ± 0.79	9.99 ± 1.5
Nickel**	0.5-1.0 (0.0005-0.001 g)	***P < 0.0001	6.67 ± 0.44	5.94 ± 1.2	3.6 ± 0.7	***P < 0.05	11 ± 0.47	9.7 ± 1.2	11.2 ± 0.86
Manganese	2-9 (0.002-0.009 g)	***P < 0.0001	447 ± 26.54	120.75 ± 41	136.56 ± 5.25	***P < 0.0001	142.78 ± 25.75	498.1 ± 72.8	282.02 ± 17.63
Iron	43 (0.043 g)	***P < 0.0001	261.8 ± 25.49	224.81 ± 30.66	155.98 ± 12.32	P > 0.05	144.49 ± 24.78	105.83 ± 81.9	152.47 ± 23.375

Table 13: Mean concentrations of the heavy metals in the gills of *C. aegyptiaca, C. parreyssi* and *C. canopicusin* g /kg at Tura and El-Kanater regions ± standard deviation.
*Permissible levels of heavy metals according to FAO/WHO, (1999).
**Permissible levels of Ni according to WHO (1989).
***Significant at P < 0.05, P < 0.0001and insignificant at P > 0.05.

Heavy metals	Tura region			P- value	El-Kanater region			P- value	*Permissible levels in mg/kg
	C. aegyptiaca	*C.canopicus*	*C. parreyssi*		*C. aegyptiaca*	*C.canopicus*	*C. parreyssi*		
Lead	0.25 (0.00025 g)	***P<0.0001	3.3 ± 0.6	3.44 ± 0.2	5.1 ± 0.54	***P< 0.05	8.45 ± 0.98	7.44 ± 1.1	6.6 ± 1.3
Copper	3 (0.003 g)	***P<0.0001	8.4 ± 0.7	6.56 ± 0.44	4.75 ± 0.24	***P<0.0001	4.9 ± 0.47	6.4 ± 0.89	7.16 ± 1
Cobalt	-	***P<0.05	1.63 ± 0.3	1.26 ± 0.3	1.1 ± 0.07	***P< 0.05	1.1 ± 0.4	2.3 ± 0.54	2.55 ± 1.17
Nickel**	0.5-1.0 (0.0005-0.001 g)	***P<0.0001	7.3 ± 1.5	5.8 ± 0.99	3.93 ± 0.6	***P<0.0001	2.7 ± 1.3	6.1 ± 0.5	7.4 ± 1
Manganese	2-9 (0.002-0.009 g)	***P<0.0001	70 ± 14.82	115.4 ± 15.35	153.5 ± 33	***P<0.0001	628.9 ± 53.7	358 ± 120.8	333.3 ± 33
Iron	43 (0.043 g)	***P<0.0001	758.7 ± 22.4	669.45 ± 27.35	453.2± 17.4	***P<0.0001	235.2 ± 93.9	911.69 ± 85	152.88 ± 13.17

Table 14: Mean concentrations of the heavy metals in the digestive tissues of *C. aegyptiaca, C. parreyssi* and *C. canopicusin* g /kg at Tura and El-Kanater regions ± standard deviation.
*Permissible levels of heavy metals according to FAO/WHO, (1999).
**Permissible levels of Ni according to WHO (1989).
***Significant at P < 0.05, P < 0.0001and insignificant at P > 0.05.

Site	Tura						El kanater					
Heavy metals	Pb	Cu	Co	Ni	Mn	Fe	Pb	Cu	Co	Ni	Mn	Fe
Water/Foot	0.007	0.002	0.043	0.01	0.003	0.0003	0.008	0.003	0.01	0.01	0.0002	0.0005
Water/Mantle	0.013	0.002	0.037	0.01	0.0001	0.0008	0.012	0.008	0.004	0.01	0.0001	0.0002
Water/Gill	0.02	0.002	0.026	0.01	0.0002	0.0013	0.006	0.005	0.001	0.01	0.0003	0.0009
Water/Digestive tissue	0.008	0.003	0.1	0.01	0.0002	0.0013	0.006	0.006	0.008	0.01	0.0003	0.0003
Sediment/Foot	0.72	0.3	0.7	0.76	1.2	0.46	0.7	0.3	4.4	1.0	1.0	0.9
Sediment/Mantle	1.4	0.3	0.6	0.44	0.5	1.3	1.0	0.8	1.4	0.6	0.36	0.4
Sediment/Gill	2.2	0.3	0.4	0.46	0.7	2.0	0.6	0.6	0.4	1.0	1.7	1.6
Sediment/Digestive tissue	0.8	0.5	1.5	0.7	0.6	2	0.5	0.7	3.4	0.93	1.53	0.54

Table 15: Mean transfer factor (TF) of the different heavy metals in the different soft parts of *C. aegyptiaca* (g/kg dry weight) and in Nile water samples (mg/l) from Tura and El-Kanater regions.

Site	Tura						El kanater					
Heavy metals	Pb	Cu	Co	Ni	Mn	Fe	Pb	Cu	Co	Ni	Mn	Fe
Water/Foot	0.004	0.002	0.05	0.1	0.0001	0.0004	0.002	0.005	0.002	0.01	0.0001	0.0003
Water/Mantle	0.007	0.003	0.02	0.01	0.0001	0.0004	0.003	0.008	0.002	0.02	0.0002	0.0004
Water/Gill	0.02	0.002	0.07	0.01	0.0004	0.0014	0.004	0.005	0.001	0.01	0.0001	0.0005
Water/Digestive tissue	0.006	0.004	0.24	0.02	0.0001	0.0009	0.009	0.004	0.006	0.01	0.0006	0.0002
Sediment/Foot	0.4	0.4	0.7	9.6	0.7	0.64	0.2	0.5	0.7	0.95	0.77	0.54
Sediment/Mantle	0.7	0.5	0.3	0.65	0.35	0.64	0.7	0.8	0.7	1.46	1.32	0.7
Sediment/Gill	2.1	0.3	1.0	0.5	1.4	1.3	0.4	0.5	0.4	0.55	0.53	0.94
Sediment/Digestive tissue	0.6	0.7	3.5	1.97	0.32	1.3	0.8	0.4	2.3	0.5	3.36	0.32

Table 16: Mean transfer factor (TF) of the different heavy metals in the different soft parts of *C. parreyssi* (g/kg dry weight) and in Nile water samples (mg/l) from Tura and El-Kanater regions.

Site	Tura						El kanater					
Heavy metals	Pb	Cu	Co	Ni	Mn	Fe	Pb	Cu	Co	Ni	Mn	Fe
Water/Foot	0.008	0.001	0.057	0.02	0.0001	0.0004	0.003	0.004	0.003	0.02	0.0002	0.0004
Water/Mantle	0.015	0.003	0.022	0.01	0.0001	0.0004	0.007	0.008	0.002	0.01	0.0001	0.0003
Water/Gill	0.017	0.001	0.05	0.01	0.0001	0.002	0.006	0.006	0.001	0.01	0.0003	0.0006
Water/Digestive tissue	0.007	0.003	0.1	0.01	0.0001	0.0002	0.009	0.005	0.007	0.01	0.0003	0.0002
Sediment/Foot	0.9	0.2	0.9	1.33	0.37	0.53	0.3	0.4	1.2	1.16	0.9	0.72
Sediment/Mantle	1.6	0.5	0.3	0.5	0.42	0.63	0.6	0.8	1.0	0.85	0.62	0.48
Sediment/Gill	1.8	0.2	0.7	0.54	0.4	0.33	0.5	0.6	0.5	0.6	1.94	1.1
Sediment/Digestive tissue	0.7	0.5	1.7	0.87	0.56	0.33	0.8	0.5	2.9	0.63	2.0	0.37

Table 17: Mean transfer factor (TF) of the different heavy metals in the different soft parts of *C. canopicus* (g/kg dry weight) and in Nile water samples (mg/l) from Tura and El-Kanater regions.

be a valuable addition to more traditional tools for determining the effects of environmental pollution on aquatic ecosystems as confirmed by Nevo et al. [42], Bickham and Smolen [43] and Nadig et al. [34].

Primer UBC 478 showed DNA alteration concerning *C. parreyssi, C. gaillardoti* and *C.canopicus*. The gain/loss of RAPD bands may be related to DNA damage, mutation or structural rearrangements induced by genotoxic agents affecting the primer sites [37]. Mutation may be due to quantitative or qualitative changes or rearrangement of

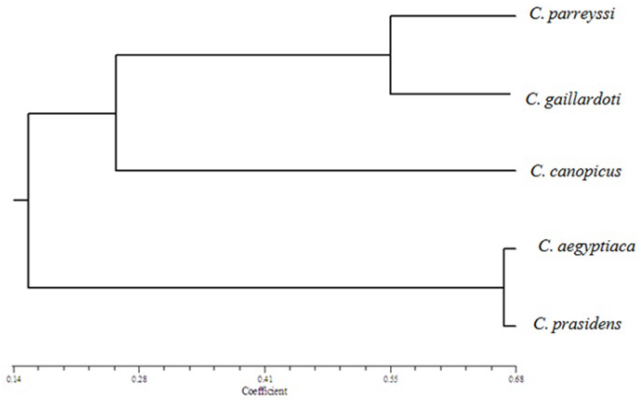

Figure 7: Dendrogram of cluster analyses of the five *Coelatura* species under investigation according to RAPD data obtained using Jaccard's coefficient and UPGMA method.

the genetic material, most probably due to metal (Pb, Mn, Cu, Fe, Co and Ni) pollution of the environment, recorded in the two localities of the study. The concept that genetic patterns within populations may be altered by exposure to contaminants was reported by Bishop and Cook (1981) [8], Klerks and Weis [9], Abdul-Aziz [44] and Giantsis et al. [38]. Also, the latter authors, examining genetic differentiation and potential impact of heavy metals pollution, using RAPD markers, observed a loss in genetic variability of *Mytilus galloprovincialis* population. They concluded that metal pollution appears to have played an important role in shapping pattern of genetic diversity and differentiation among Greek *M. galloprovincialis* population. Yap et al. [45] found that heavy metal contamination was a main causal agent for the genetic differentiation of *Pera viridis* in Peninsula Malaysia. The evidence for pollutant to induce genotoxicity has been also determined by several authors [33,38,46-50]. Neeratanaphan et al. [51] postulated that understanding the effect of heavy metals on genetic variability is fundamental for preservation. They detected genomic DNA modification such as damage and structure variation in the freshwater snail *Filopaludina martensi* affected by lead and cadmium

Forni [52] and Reid et al. [53] reported Cu, Fe, Cd and Ni as mutagenic agents. These metals have the tendency to bind to phosphates and wide variety of organic molecules including base residues of DNA, which can lead to mutations by altering structures of DNA [54] or modifying the genetic diversity of populations. Also, exposure of mussels in the field to water polluted by different mixtures of genotoxic contaminants was reported by Izquierdo et al. [55] to induce DNA alterations, leading to genetic variation among species and populations. Thus, the conflict in the taxonomy of *Coelatura* species in the different studies is most probably due to the environmental pollution with heavy metals among other factors. Heavy metals analysed in all tissues of the studied *Coelatura* species exceeded the permissible levels according to WHO (1989) [28] and FAO/WHO [29].

The numbers of threatened aquatic species and species extinctions increase at an alarming rate [56]. Molluscs are one of the most threatened major taxonomic groups worldwide [57]. Within this group, the unionids are highly threatened throughout their distribution [58] and are declining globally due to alteration in habitat, decline and extinction of fish host populations, pollution and environmental changes, pointing toward impending extinction. They are the most imperiled group of species and many species became extinct in several parts of the world including Egypt, while others are threatened or endangered. The loss of benthic biomass may result in large scale alterations of freshwater ecosystem processes and functions [59].

Little information is available about the effect of frequent exposure to metals on mussels; it is possible that higher metal amounts than required could be a contributing factor to the extinction of some mussel species and genetic variation of some other species. In fact, the mussel fauna in Egypt is threatened due to heavy metal pollution of the River Nile water and sediment among other factors.

All species of genus *Unio* which were used to live in the River Nile are today extinct [10]. Only fossils were recorded by these authors from El Fayoum, Komombo, Idfu and Isna i.e from Upper Egypt. Although, some investigators [60,61] have referred to living *Unio* specimens in the River Nile in Lower Egypt. But, this is uncertain and needs to be thoroughly revised and the occurrence of *Unio* species in Egypt is still doubtful.

In general, studies on heavy metals are important in two main aspects, the public health point of view and the aquatic environment conservation. Heavy metals are present in the aquatic environment where they can accumulate along the food chain. Moreover, small amounts of absorbed heavy metals are either stored in a metabolically available form for essential biochemical processes or detoxified into metabolically inert forms and held in the body either temporarily or permanently [62-64]. Thus, determination of chemical quality of aquatic organisms, particularly the content of heavy metals is extremely important.

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