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Impact of the size of commercial bivalves on bioaccumulation and depuration of heavy metals

Mahmoud Sami[*], **Nesreen K. Ibrahim, Deyaaedin A. Mohammad**
Department of Marine Science , Faculty of Science, Suez Canal University, Egypt.

***Corresponding Author: Mahmoud_Sami@science.suez.edu.eg**

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ABSTRACT

The edible clams from Lake Timsah are exposed to different industrial wastes which may reflect the reason for the high concentration of heavy metals in studied species. The current study aims to evaluate the effect of size classes on the elimination of heavy metals (Cu, Fe, Pb, Co, Ni, and Zn) in some commercial bivalves *Ruditapes decussatus*, *Venerupis pullastra* and *Paphia undulata*. Negative correlations were found between the sizes of studied species for all heavy metals (except Cu which showed a positive correlation with size in *V. pullastra*). The concentrations of all heavy metals (Cu, Fe, Pb, Co, and Zn) in the studied species were higher than those in water and sediment. The highest depuration rate for all studied species was recorded in small clam classes.

INTRODUCTION

The coastal areas and estuaries are the most exposed areas to chemical pollution. **(Almeida and Soares, 2012)**. In many countries, increasing of the industrialization and agricultural activities contribute to an increasing of discharge of chemical pollutants into the ecosystem, which lead to increase in metals levels in the natural waters that causing damage of fresh and marine habitats **(Muhammad *et al.*, 2011; El Nemr, 2012)**. Trace metals exist naturally in the earth's crust. Some of them are essential for biological systems, since they participate in numerous enzymatic processes **(Pellerin and Amiard, 2009)**. On the other hand, trace metals such as cadmium, lead and mercury are generally toxic for organisms, even at low concentrations **(Stankovic and Jovic, 2012)**.Their bioaccumulation in tissues lead to intoxication, decreased fertility, cellular and tissue damage, cell death, dysfunction of a variety of organs and induce cancer in humans **(Benavides *et al.*, 2005; Nordberg, 2010)**.

Bivalve molluscs can take up contaminants from sediments, suspended particulate materials, water column and also food sources **(Laffon *et al.*, 2006)**. They have been widely used for many years as bioindicator organisms in monitoring of chemical pollutants and biomonitoring in aquatic ecosystems. This is particularly due to their sedentary nature or immobility, filter-feeding activity, low metabolism, contact with sediments, wide distribution in all environments, ability to bioaccumulate pollutants and high tolerance to chemical exposure due to a remarkably active immune system **(Waykar and Deshmukh, 2012; Zuykov *et al.*, 2013)**.

The bioaccumulation rate of metals in bivalves depends on biotic factors (e.g. species, age, sex, soft-body weight, gametogenesis and physiological status) and abiotic factors (e.g. availability of contaminants in the environment, filtration rate, temperature, salinity, pH, chemical species and interaction with other elements) **(Fernandez- Tajes *et al.*, 2011)**. Venerid clams represent the most commercially important and successful group of bivalves in Suez Canal particularly in Lake Timsah and the Bitter Lakes **(Mohammed *et al.*, 1992)**. In Egypt, these clams are greatly appreciated by seafood consumers. Moreover, the clam industry has suffered a decline in sales in the local market as a result of heightened publicity being given to clam-related illnesses. Heavy metals, among other contaminants, are present in high concentrations in industrial effluents discharged into Lake Timsah **(Ibrahim and Abu El- Regal, 2014; Marwa and El-HaK, 2017)**.

There are two types of depuration:1) Facility based depuration, 2) Relay depuration. The first type depends on moving the contaminated shellfish to land based depuration facility where the shellfish is extensively cleansed with clean water. However, this process is expensive and causing stress to the shellfish. The second type relies on natural cleaning process that occurs when the contaminated shellfish transferred to approved or cleaned water **(Chalek, 2013)**.

Depuration rates of shellfish impacted by many factors including size, siphoning activity, physiological conditions **(Richards, 1988; Jones *et al.*, 1991)**, type and amount of contamination, water quality parameters such as temperature and salinity **(Chalek, 2013)**, shellfish to water ratio, flow rate, oxygenation, species and duration **(jones *et al.*, 1991; Barile *et al.*, 2009; Cozzi *et al.*, 2009; Lee *et al.*, 2010; Anacleto, 2014)**.

Most studies concerning depuration exposed bivalves to pollutants in the laboratory and then transferred them to clean waters under laboratory or field conditions **(Wahi *et al.*, 2009)**. A few studies used clams containing naturally high concentrations of heavy metals and followed their depuration at a relatively clean field **(El-Shenawy, 2004)**. No information was reported on the impact of different parameters (clam size, temperature and etc….) on depuration and survival of the commercial clams. The current study aims to evaluate the effect of clam size on elimination of heavy metals (Cu, Fe, Pb, Co, Ni and Zn) in some commercial bivalves *Ruditapes decussatus*, *Venerupis pullastra* and *Paphia undulat*a.

MATERIALS AND METHODS

Sample collection and set up

The experiment was performed in the Mariculture laboratory, at the Department of Marine Science, Suez Canal University, Ismailia. The clam species *R. decussatus, V. pullastra* and *P. undulata* were collected from their location in Lake Timsah in February 2019. In the laboratory, samples of clams were first cleaned to eliminate any debris and then splashed by distilled water. Their shell lengths were measured by a digital Vernier caliper with an accuracy of 0.01 mm. The edible part of the meat was carefully removed by shelling the samples with a plastic knife. The soft tissues were placed only in plastic bags and frozen until it was examined. Samples of water and sediment were collected from the same site to determine the initial level of heavy metals. Water samples were collected from a precise depth corresponding to the bivalve settlements. Sediment samples were collected and dried to a constant weight at 80°C and then stored in polyethylene bags until analysis.

Depuration experiments

The depuration experiments were commenced within 4 h of shellfish collection. The water that used in all experiments is synthetic with salinity 25 ‰ and the water changed in all tanks twice a day to avoid absorption of depurated contaminants. Before the start of depuration experiments, the heavy metals (Cu, Fe, Pb, Co, Ni and Zn) in all sizes classes of clams were measured (0 day or before depuration). Survival of clams throughout the treatments was monitored daily. Shells that were found open but did not close when touched were considered dead and therefore removed. Number of dead clams was recorded and computed into mortality rates.

Depuration experiment: Thirty plastic tanks (3 L) were used. The experiment was implemented at constant temperature (15 ±1 ºc). The experiments were carried out in three replicates for each clam size with 3 days depuration time. *R. decussatus* divided into four size classes (15-20, 20-25, 25-30 and 30-35 mm) while *V. pullastra* and *P. undulata* divided into three size classes (15-20, 20-25 and 25-30) and (20-25, 25-30 and 30-35 mm), respectively.

Metal Analysis

The results for metal concentration are expressed as milligrams per liter (ppm), micrograms per gram of dry weight, and micrograms per gram of dry weight in water, clam tissue, and sediment, respectively.

Determination of heavy metals concentration in bottom sediment

In order to detect heavy metals contamination in sediment samples, an exact weight of the dry sediment sample (0.5 g) was fully digested in Teflon vessels using mixture of concentrated acids (analar nitric acid (HNO_3) and $HCLO_4$) for about 2 hours (triplicate digestion were made for each sample). The final solution was diluted to 25 ml with distilled de- ionized water **(Oregioni and Astone, 1984)**. Their metal content was measured by AAS described by **Usero *et al.* (2005)**.

Determination of heavy metals concentration in water

Water samples were obtained and filtered through a 0.45 µm membrane in one liter of white polyethylene bottles. The concentration of the following heavy metals: Cu, Fe, Pb, Co, Ni and Zn were determined by digesting 100 of each water sample at 100 ºC in 5 ml analar grade nitric acid (HNO_3) for 5 hours. The digested samples were allowed to stand overnight at room temperature before the heavy metal residual was analyzed in accordance with Standard method 3110 **(APHA, 1992)**.

Determination of heavy metals concentration in soft parts of studied species

The soft tissues were dried 12 hours before examination at 70 °C. The analytical procedure used to measure the metals Cu, Fe, Pb, Co, Ni and Zn was based on **UNEP/FAO/IAEA (1982)** with modification as follows: sub-sample (dried) tissue (0.5 g) was heated with 10 ml of concentrated nitric acid (70 to 90°C) till all tissue had been digested. The temperature was then gradually increased to 135 °C and drops of H_2O_2 added for further oxidation. After cooling, solutions were diluted to 50 ml with double distilled water and filtered with 1.6µm fiberglass filter paper (GF/A). Samples were then stored at room temperature in 50 ml volumetric flask until they were analyzed. Analyses were carried out with flame Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer 2380, Faculty of Science, Ismailia, Egypt) with electrode discharge lamps (EDL) and hollow cathode lamp (HCL).

Metal pollution index

To compare the total content of metals of the three different clam species, Metal Pollution Index (MPI) was used, obtained with the equation **(Usero *et al*., 1997)**: where,

$$\mathbf{MPI = (Cf1,\ x\ Cf2,\ ...\ Cfn)^{1/n}\quad [1]}$$

Cfi = concentration factor for the metal i in the sample.

n= number of metals.

Bioaccumulation factor (BAF) and bioaccumulation sediment factor (BASF)

The distribution behavior of heavy metals between water and biota can be expressed as a bioaccumulation factor (BAF) **(Chevereuil *et al.*, 1996)**.To evaluate the efficiency of metal bioaccumulation in bivalves, the bioaccumulation sediment factor (BASF) was calculated as a ratio of the average bivalve metal to the average sediment concentration at a given time **(Zhao *et al.* 2012)**.

$$BAF=\frac{CX}{CW}\qquad [2]$$

Where CX: mean metal concentrationin clams,

CW: mean concentration metal in water

$$BASF=\frac{CX}{CS}\qquad [3]$$

Where CX: mean metal concentration in clams,

CS: mean metal concentration in sediment

Metal depuration rate ($\mu g/g\ day^{-1}$)

The rate of metal depuration was calculated according to the following formula **(Yap *et al.*, 2003)**:

$$\textbf{Metal depuration rate} = \frac{\text{Metal level before depuration} - \text{Metal levelafter depuration}}{\text{Days of depuration}} \quad [4]$$

Metal depuration percentage or reduction rate (%)

$$\textbf{Reduction rate (\%)} = \frac{\text{Metal level before depuration} - \text{Metal levelafter depuration}}{\text{Metal level before depuration}} \times 100 \quad [5]$$

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS (Version 22). One-way Analysis of variance (ANOVA) used to test the significant differences between concentration of heavy metals in different studied species, water, and sediment and between the zero and third day of depuration. If significant differences were present, Tukey's HSD test was employed to check for differences between means. Relationships between size classes and metal concentration and reduction rates were performed through the correlation matrix. Significance levels for all analysis were set at $p<0.05$.

RESULTS

1. Initial concentration of heavy metals in water, sediments and organisms:

The concentrations of heavy metals in water, sediment and soft body of studied species are presented in tables 1 and 2. The concentration of all heavy metals (Cu, Fe, Pb, Co and Zn) in the studied species was higher than their concentration in water and sediment. However, nickel reading showed high values in sediment compared to water and organisms.

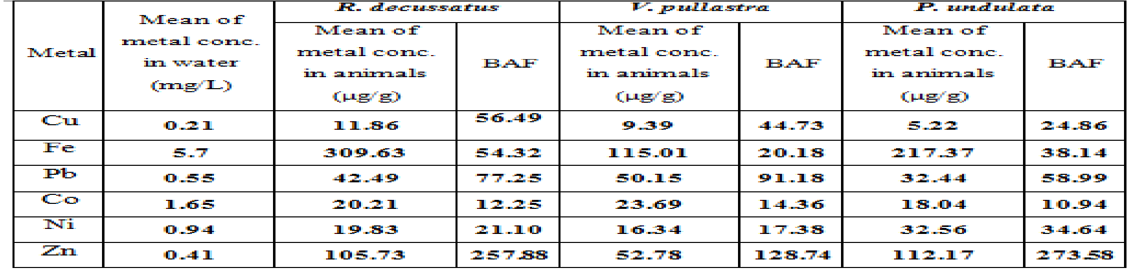
Fe was considered the most dominant metal in water, sediment and the studied species while Cu was the lowest concentration. The descending order of heavy metals was: Fe > Co > Ni>Pb>Zn >Cu; Fe > Zn > Ni>Co> Pb>Cu; Fe > Zn > Pb>Co >Ni >Cu; Fe > Zn > Ni >Pb >Co> Cu; Fe > Zn > Pb>Co >Ni >Cu for water, sediment, *R. decussatus, P. undulata and V. pullastra*, respectively.

Generally, there was significant difference in the concentration of heavy metals between the three studied species ($p<0.01$). The highest concentration of Cu and Fe recorded in *R. decussatus* while Pb and Co recorded highest values in *V. pullastra*. On other hand Ni and Zn recorded greatest values in *P. undulata*. *R. decusstaus* had the highest metal pollution index (MPI) while the lowest MPI was recorded in *V. pullastra* (Table 3).

The values of bioaccumulation factor (BAF) are summarized in Table 1. The order of BAF according to highest concentration or descending was: Zn > Pb > Cu>Fe>Ni >Co; Zn > Pb > Fe>Ni>Cu >Co; Zn > Pb > Cu>Fe>Ni >Co for *R. decussatus, P. undulata*and *V. pullastra*, respectively. Table 2 shows the values of bioaccumulation sediment factor

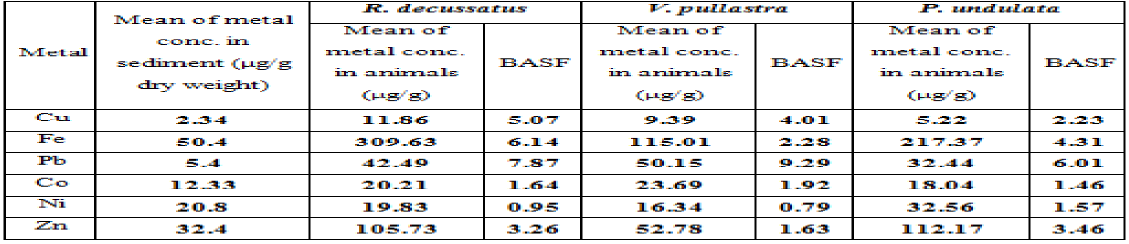
(BSAF). BASF arranged in the following order: Fe > Zn > Pb>Co>Ni >Cu; Fe > Zn > Ni>Pb>Co >Cu; Pb > Cu > Fe>Co>Zn >Ni for *R. decussatus*, *P. undulata* and *V. pullastra*, respectively.

Table 1: Mean concentration of heavy metals in water, studied species and its bioaccumulation factor (BAF).



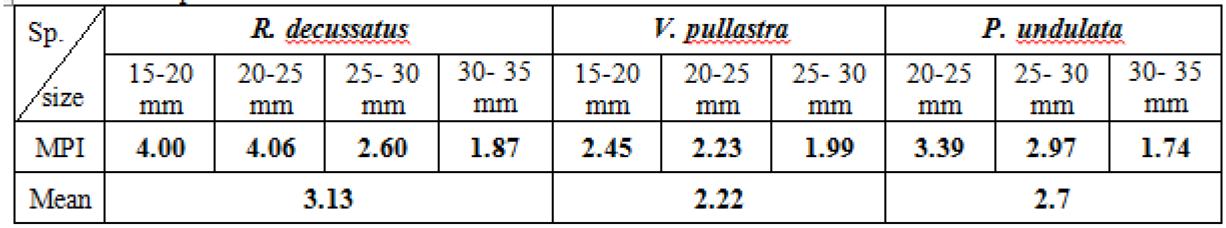
Metal	Mean of metal conc. in water (mg/L)	*R. decussatus*		*V. pullastra*		*P. undulata*	
		Mean of metal conc. in animals (µg/g)	BAF	Mean of metal conc. in animals (µg/g)	BAF	Mean of metal conc. in animals (µg/g)	BAF
Cu	0.21	11.86	56.49	9.39	44.73	5.22	24.86
Fe	5.7	309.63	54.32	115.01	20.18	217.37	38.14
Pb	0.55	42.49	77.25	50.15	91.18	32.44	58.99
Co	1.65	20.21	12.25	23.69	14.36	18.04	10.94
Ni	0.94	19.83	21.10	16.34	17.38	32.56	34.64
Zn	0.41	105.73	257.88	52.78	128.74	112.17	273.58

Table2: Mean concentration of heavy metals in sediment, studied species and its bioaccumulation sediment factor (BASF).



Metal	Mean of metal conc. in sediment (µg/g dry weight)	*R. decussatus*		*V. pullastra*		*P. undulata*	
		Mean of metal conc. in animals (µg/g)	BASF	Mean of metal conc. in animals (µg/g)	BASF	Mean of metal conc. in animals (µg/g)	BASF
Cu	2.34	11.86	5.07	9.39	4.01	5.22	2.23
Fe	50.4	309.63	6.14	115.01	2.28	217.37	4.31
Pb	5.4	42.49	7.87	50.15	9.29	32.44	6.01
Co	12.33	20.21	1.64	23.69	1.92	18.04	1.46
Ni	20.8	19.83	0.95	16.34	0.79	32.56	1.57
Zn	32.4	105.73	3.26	52.78	1.63	112.17	3.46

Table 3: Metal pollution index (MPI) for total heavy metals in different size classes of the studied species.



Sp. / size	*R. decussatus*				*V. pullastra*			*P. undulata*		
	15-20 mm	20-25 mm	25- 30 mm	30- 35 mm	15-20 mm	20-25 mm	25- 30 mm	20-25 mm	25- 30 mm	30- 35 mm
MPI	**4.00**	**4.06**	**2.60**	**1.87**	**2.45**	**2.23**	**1.99**	**3.39**	**2.97**	**1.74**
Mean	**3.13**				**2.22**			**2.7**		

2. Initial concentration of heavy metals in different size clams:

The initial concentrations of heavy metals (with different size classes) for studied species are illustrated in Figs. 1-3. The size classes 20-25mm, 20-25 mm and 15-20 mm had the highest MPI for *R. decussatus*, *P. undulata* and *V. pullastra*, respectively, while the lowest values of MPI recorded in 30-35 mm size class for *R. decussatus*, and *P. undulate* and 25-30 mm for *V. pullastra* (Table 3).

Table 4 shows Pearson correlation between metal concentrations and different molluscan size classes. Negative correlations were found between the size of *R. decussatus*, *P. undulata*and *V. pullastra* for all heavy metals except Cu and Fe which showed positive correlation with length for *V. pullastra* only. In *R. decussatus*, there was significant correlation in length or size classes with Cu, Fe, Pb and Zn.*P. undulata* had significant correlation in all metals except Co and Zn, while *V. pullastra* had significant correlation with Fe, Cu and Zn(Table 4).

Table 4: Pearson correlation coefficient (r) between metal concentrations and clam size

Species	metals	r
R. decussatus	Cu	-0.706*
	Fe	-0.624*
	Pb	-0.893**
	Co	-0.526
	Ni	-0.397
	Zn	-0.842**
P. undulata	Cu	-0.813**
	Fe	-0.949**
	Pb	-0.775*
	Co	-0.339
	Ni	-0.991**
	Zn	-0.386
V. pullastra	Cu	0.733*
	Fe	0.794*
	Pb	-0.638
	Co	-0.437
	Ni	-0.259
	Zn	-0.771*

Significant level: *= $p<0.05$; **= $p<0.01$

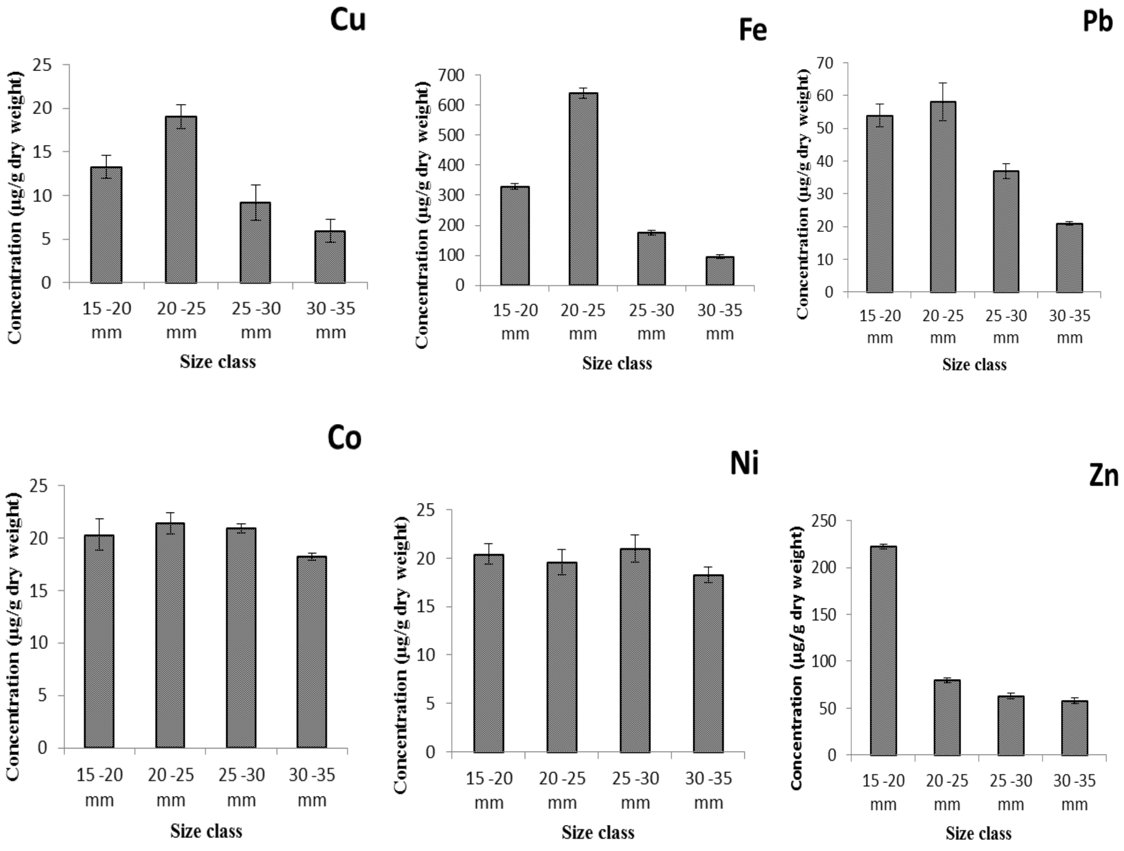


Fig.1: Concentration of heavy metals in the different size classes of *Ruditapes decussatus*

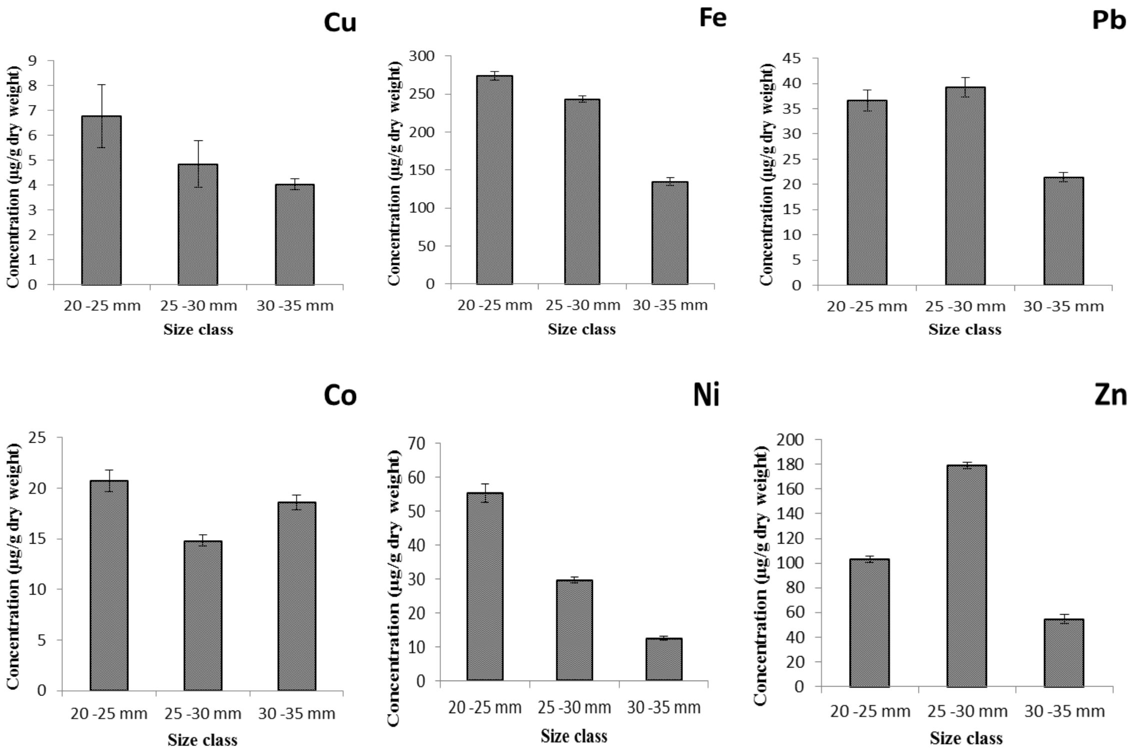


Fig. 2: Concentration of heavy metals in the different size classes of *Paphia undulata*

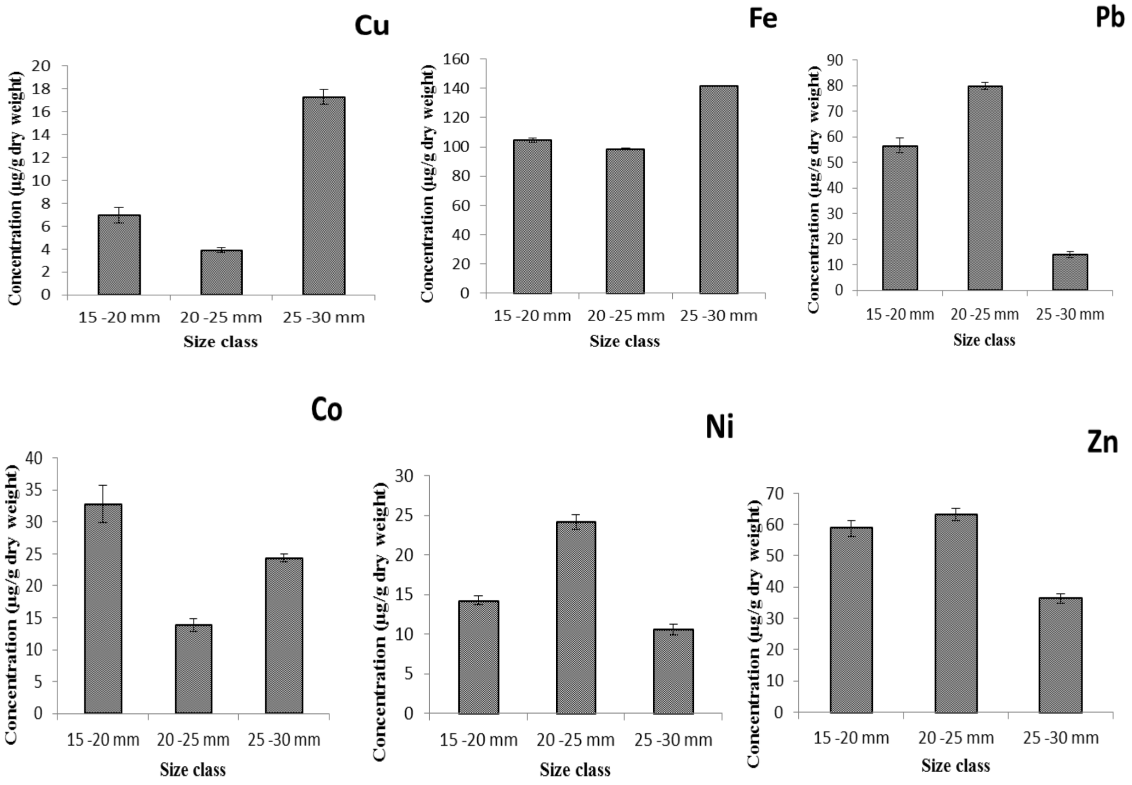
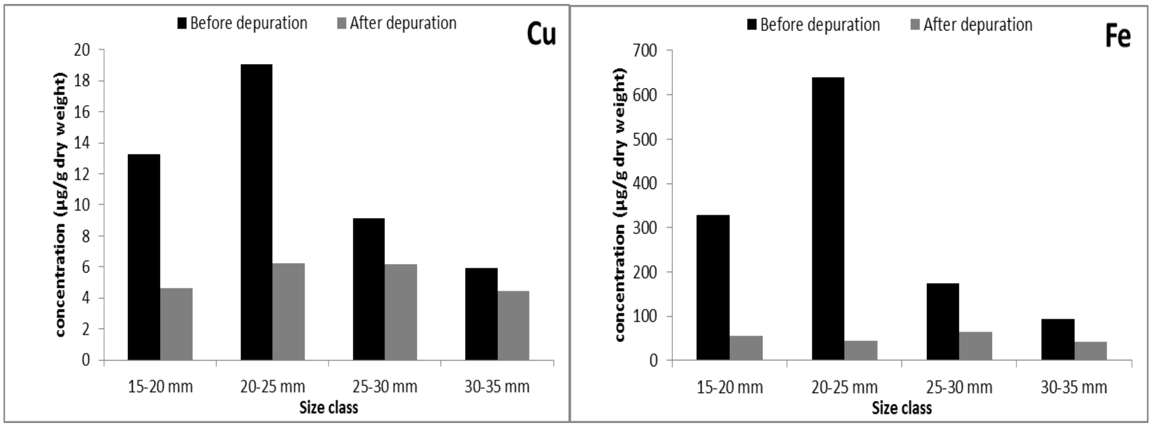


Fig.3: Concentration of heavy metals in the different size classes of *Venerupis pullastra.*

3. Depuration Experiment

The concentration of heavy metals before and after depuration with different size classes for studied species are presented or showed in Figs. 4- 6. The concentration of all heavy metals for studied species after 3 days of depuration were significantly decreased compared with initial concentration(p<0.05). There was significant difference between the depuration rate of all heavy metals for all studied species (P<0.01). The highest depuration rate or the faster reduction for concentration of Cu, Fe, Pb, Co, Ni and Zn rate were recorded at size classes 20-25 mm, 20-25 mm, 20-25 mm, 25-30mm, 25-30mm and 15-20mm, respectively, for *R. decussatus* while the lowest values for all heavy metals were recorded at size class 30-35 mm for *R. decussatus* (Tables5 and 6).The highest depuration rate for Cu, Pb, and Zn were recorded at sizes classes 25-30 mm for *P. undulata* while the highest depuration rate for Fe, Co and Ni were recorded at size class 20- 25 mm for *P. undulata.*The lowest values for all heavy metals were recorded at size class 30-35 mm for *P. undulata.* The highest depuration rate for Fe, Pb, Ni and Zn rate were recorded at sizes classes 20-25 mm for *V. pullastra*. The highest depuration rate or the faster reduction for Cu and Co were recorded at size class 25- 30 mm and 15-20 mm, respectively, for *V. pullastra.* The lowest values for all heavy metals were recorded at size class 25-30 mm for *V. pullastra* except Cu and Co (20-25mm size class) (Tables 5 and 6).

Table 7 showed the Pearson correlation between metal depuration rate and different bivalve size classes. Negative correlations were found between the sizes of studied species for all heavy metals (except Cu which showed positive correlation with size in *V. pullastra*). The smallest animals had high depuration rate compared with the large animals. In *R. decussatus*, there was significant correlation in length or size classes with depuration rates of Cu, Fe, Pb and Zn.*P. undulata* had significant correlation in all metals (except Cu and Zn). However, *V. pullastra* had no significant correlation with all metals (except Cu and Co) (Table 7).



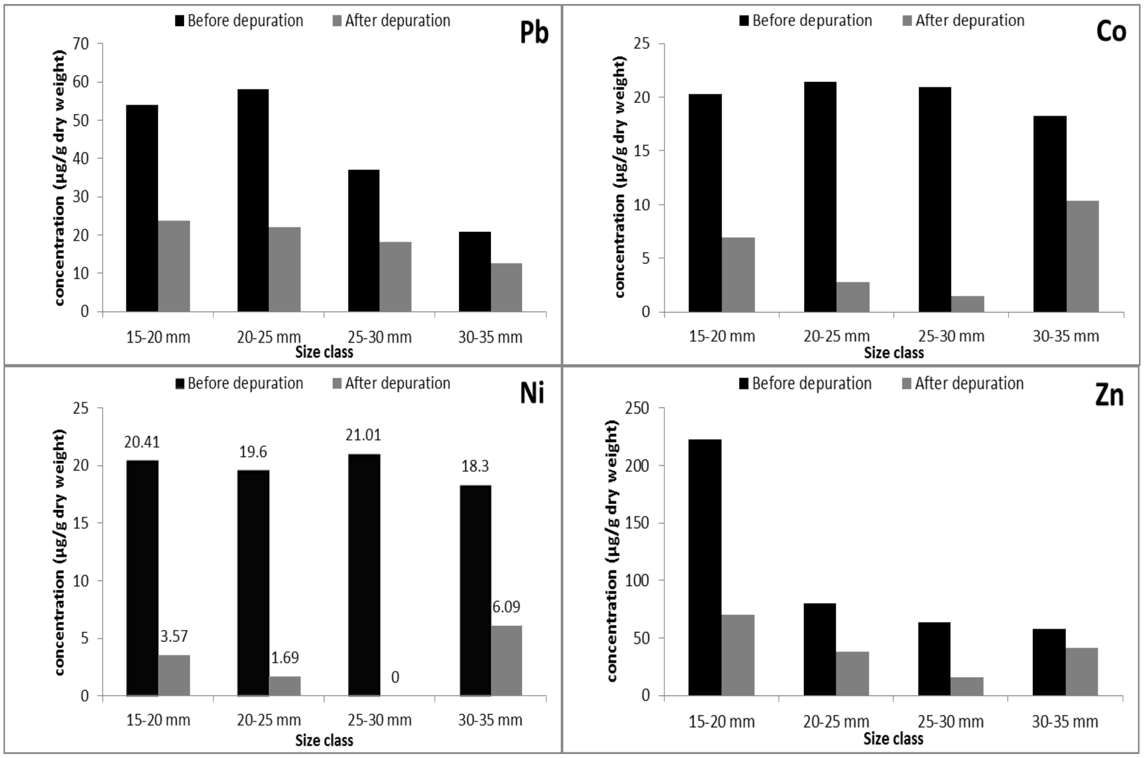
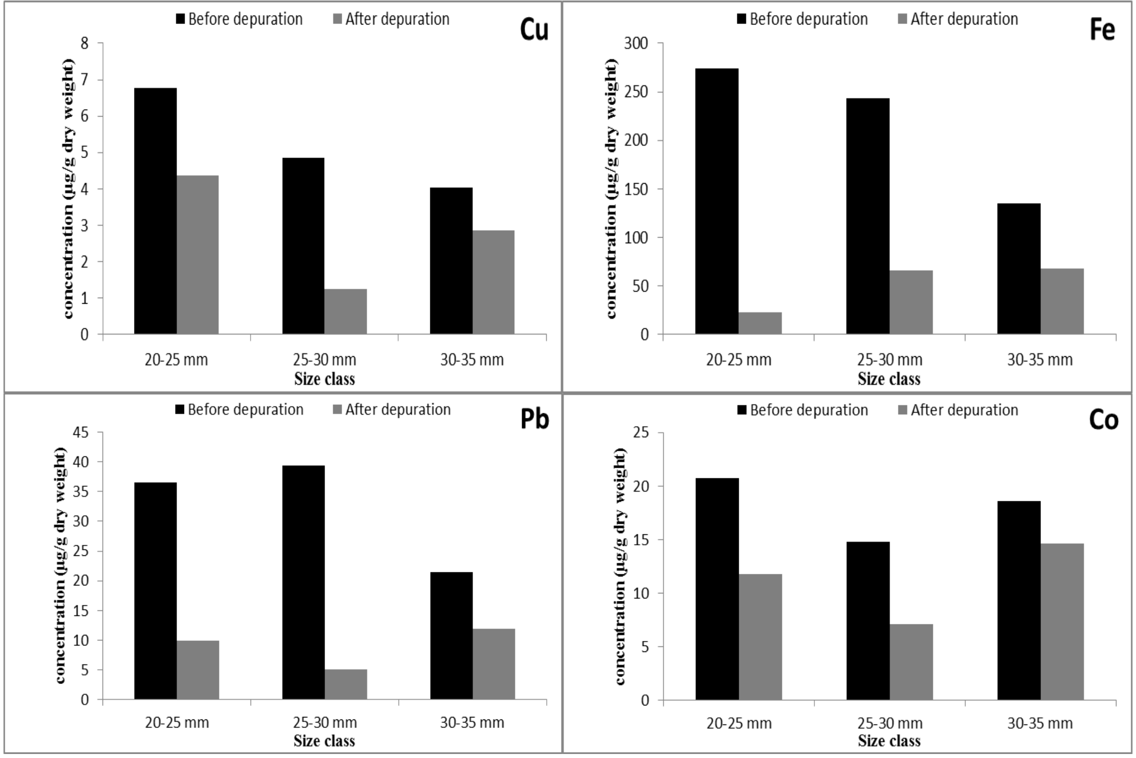


Fig. 4: Mean concentration of heavy metals in different size classes of *R. decussatus* before and after 3 days – depuration.



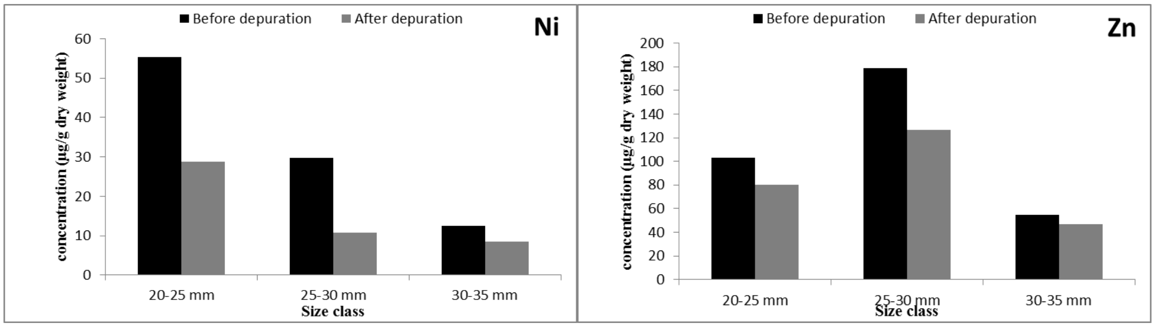


Fig. 5: Mean concentration of heavy metals in different size classes of *P. undulata* before and after 3 days – depuration.

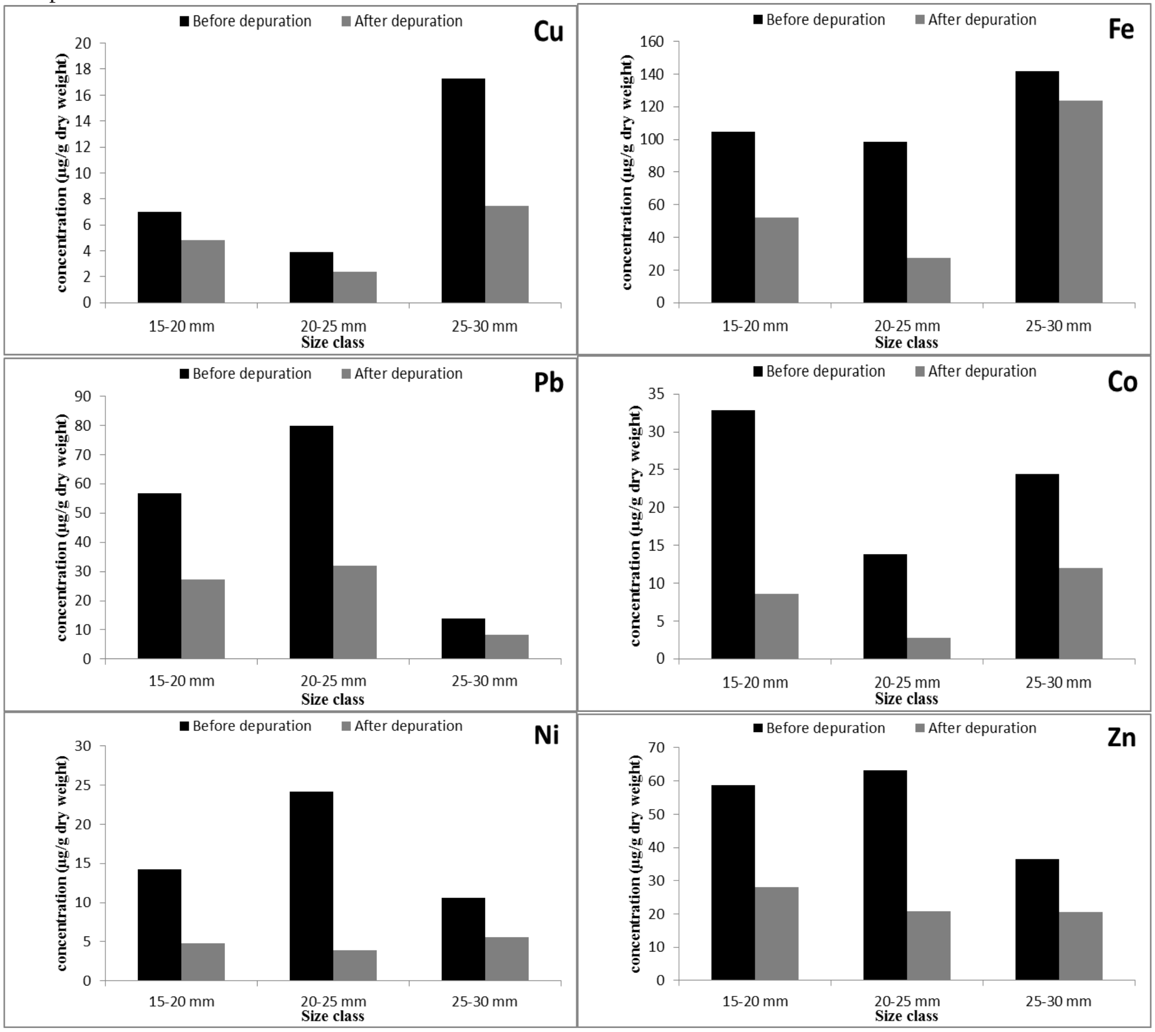
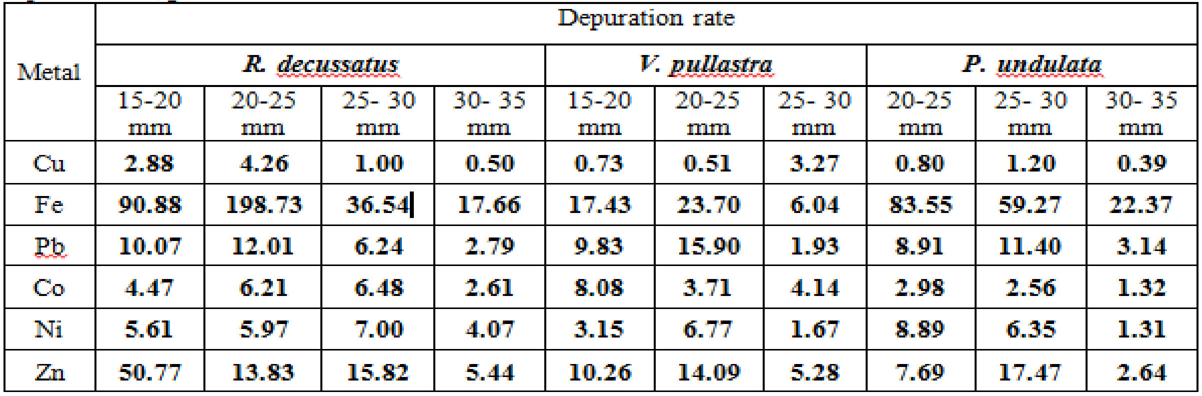


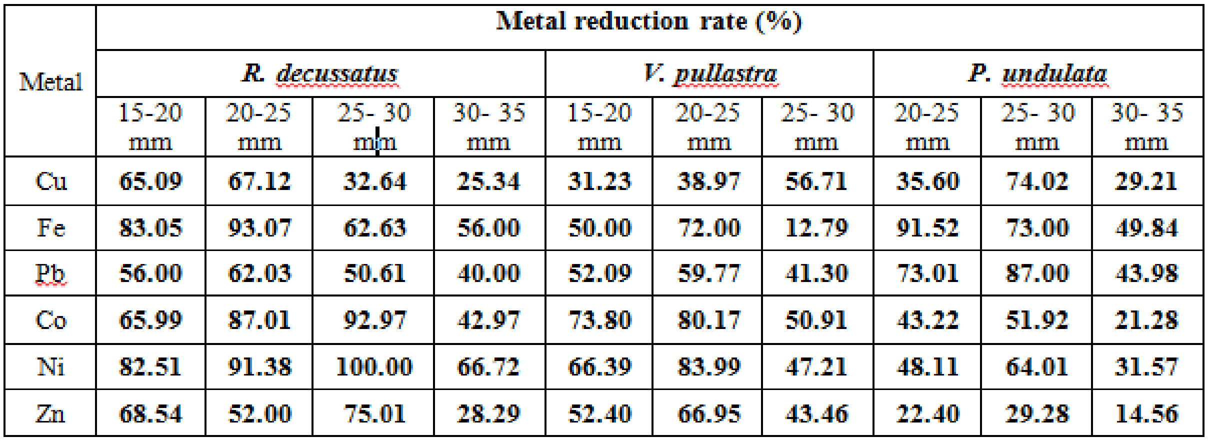
Fig. 6: Mean concentration of heavy metals in different size classes of *V. Pullastra* before and after 3 days – depuration.

Table 5: Depuration rate (µg/g dry weight day^{-1}) of heavy metals in the soft tissues of studied species through different size classes.



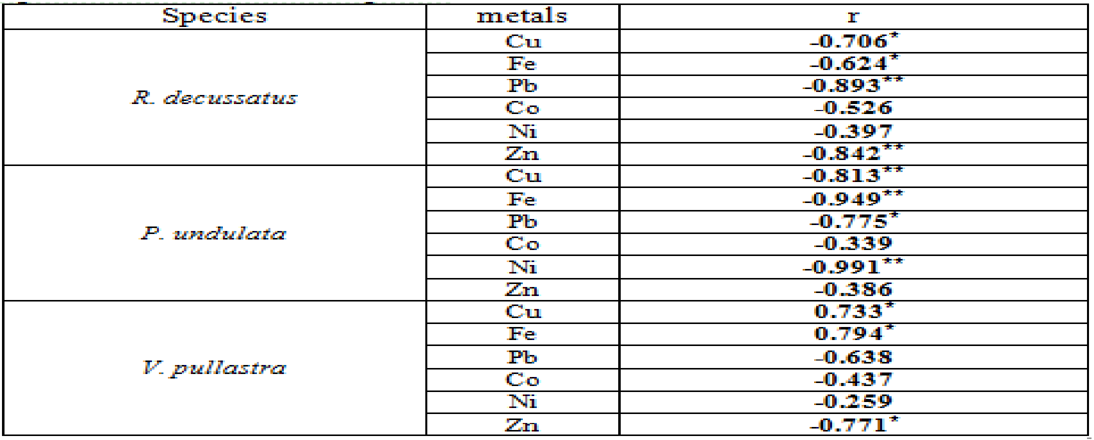
Metal	Depuration rate									
	R. decussatus				***V. pullastra***			***P. undulata***		
	15-20 mm	20-25 mm	25- 30 mm	30- 35 mm	15-20 mm	20-25 mm	25- 30 mm	20-25 mm	25- 30 mm	30- 35 mm
Cu	**2.88**	**4.26**	**1.00**	**0.50**	**0.73**	**0.51**	**3.27**	**0.80**	**1.20**	**0.39**
Fe	**90.88**	**198.73**	**36.54**	**17.66**	**17.43**	**23.70**	**6.04**	**83.55**	**59.27**	**22.37**
Pb	**10.07**	**12.01**	**6.24**	**2.79**	**9.83**	**15.90**	**1.93**	**8.91**	**11.40**	**3.14**
Co	**4.47**	**6.21**	**6.48**	**2.61**	**8.08**	**3.71**	**4.14**	**2.98**	**2.56**	**1.32**
Ni	**5.61**	**5.97**	**7.00**	**4.07**	**3.15**	**6.77**	**1.67**	**8.89**	**6.35**	**1.31**
Zn	**50.77**	**13.83**	**15.82**	**5.44**	**10.26**	**14.09**	**5.28**	**7.69**	**17.47**	**2.64**

Table 6: Reduction rate of heavy metals in the soft tissues of studied species through different size classes.



Metal	**Metal reduction rate (%)**									
	R. decussatus				***V. pullastra***			***P. undulata***		
	15-20 mm	20-25 mm	25- 30 mm	30- 35 mm	15-20 mm	20-25 mm	25- 30 mm	20-25 mm	25- 30 mm	30- 35 mm
Cu	**65.09**	**67.12**	**32.64**	**25.34**	**31.23**	**38.97**	**56.71**	**35.60**	**74.02**	**29.21**
Fe	**83.05**	**93.07**	**62.63**	**56.00**	**50.00**	**72.00**	**12.79**	**91.52**	**73.00**	**49.84**
Pb	**56.00**	**62.03**	**50.61**	**40.00**	**52.09**	**59.77**	**41.30**	**73.01**	**87.00**	**43.98**
Co	**65.99**	**87.01**	**92.97**	**42.97**	**73.80**	**80.17**	**50.91**	**43.22**	**51.92**	**21.28**
Ni	**82.51**	**91.38**	**100.00**	**66.72**	**66.39**	**83.99**	**47.21**	**48.11**	**64.01**	**31.57**
Zn	**68.54**	**52.00**	**75.01**	**28.29**	**52.40**	**66.95**	**43.46**	**22.40**	**29.28**	**14.56**

Table 7: Pearson correlation coefficient among lengths and depuration rates (µg/g dry weight day^{-1}) of heavy metals in the studied species and its significance values.



Species	metals	r
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	Fe	**-0.624***
	Pb	**-0.893****
	Co	**-0.526**
	Ni	**-0.397**
	Zn	**-0.842****
P. undulata	**Cu**	**-0.813****
	Fe	**-0.949****
	Pb	**-0.775***
	Co	**-0.339**
	Ni	**-0.991****
	Zn	**-0.386**
V. pullastra	**Cu**	**0.733***
	Fe	**0.794***
	Pb	**-0.638**
	Co	**-0.437**
	Ni	**-0.259**
	Zn	**-0.771***

Significant level: *= $p<0.05$; **= $p<0.01$

DISCUSSION

During the present study, three species of edible clams were collected from Lake Timsah, Suez Canal, Egypt. This site is exposed to different industrial wastes which may reflect the reason of the high concentration of heavy metals in studied species. This confirms the fact that clams are the most reliable tool for identifying sources of biological available heavy metals **(Yap *et al.*, 2009)**.

There are many factors which influence trace metal accumulation in animals such as salinity and temperature **(Phillips, 1976)**. Other factors influencing bioaccumulation of trace metals include life stage of organisms and mode of feeding **(Okazaki and Pamietz, 1981)**. Factors especially important in decreasing bioaccumulation of heavy metals are low pH, low temperature, and high organic content of the substrate. The temperature and pH effects might be explained by increased stress at reduced temperature and pH, resulting in reduced food intake and/or diminished mucus secretion in the gills **(Elder and Collins, 1991)**.

The present work showed that Fe and Zn were the most abundant metals in the sediments. High Fe and Zn concentration is related to its wide distribution in the environment, since most rocks and minerals contain Zn and natural and anthropogenic activities result in the transport of this metal through the atmosphere, water and soil **(Fatoki and Awofolu, 2003)**. The cuurent results are in agreement with the previous studies **(Ibrahim and Abu El-Regal, 2014; AbdElGhany, 2017; Mohammad *et al.*, 2017)**. **Gabr and Gab-Alla (2008)** found that Zn was the high concentration in Suez Canal while the low concentrations were recorded for Fe. This result disagrees with the present study where the most dominant metal in sediment was Fe. The concentrations of Cu, Pb, Co and Zn in the present study were less than **Gabr and Gab-Alla (2008)** results at the same area.

The copper concentration in sediment in the present study were less than its values in the previous study **(El- Shenawy *et al.*, 2016; AbdElGhany, 2017; Mohammad *et al.*, 2017)** while its concentration was higher than **El-Gamal (2011)**. The iron concentration was higher than **Gabr and Gab-Alla (2008); Ibrahim and Abu El-Regal (2014); AbdElGhany (2017)** results while it was less than **El- shenawy *et al.* (2016); Mohammad *et al.* (2017)**. The value of Pb in sediment was less than its values in the previous studies **(Ibrahim and Abu El-Regal, 2014; El- Shenawy *et al.*, 2016; Abdelghany, 2017); Mohammad *et al.*, 2017)**.

Gabr and Gab-Alla (2008); El Gamal (2011), **Ibrahim and Abu El-Regal(2014); El-Shenawy *et al.* (2016)** found that the concentration of all heavy metals was high in sediment than water. Their results are agreement with the present results. The high concentration of heavy metals in sediment, revealed that the metals precipitate in sediment than its dissolving in water. The concentration of Cu, Fe and Pb in water were high in the present study than its value in the previous studies (**Gabr and Gab-Alla, 2008; Saad El-Din *et al*., 2014**). While their concentration was less than **El Khodary *et al*. (2018); Salama *et al*. (2020)** results. The differences of the concentration of heavy metal between the present study and the previous studies may be attributed to different sources of pollution and its amount and different study sites or location..

The permissible limit for heavy metals differs from organization to another and from country to another. Pb is the second element on the top 20 list of the most poisoning heavy metals. Its target organs are bones, brain, blood, kidneys, thyroid gland, reproductive and cardiovascular systems **(Massadeh *et al.*, 2004)**. By comparing the data of the present study (Table 1) to permissible limits on dry weight basis, it was observed that lead (Pb) concentration (av. 42.49, 32.44 and50.15 µg/g d.wt.) for *R. decussatus, P. undulat*a and *V. pullastra*, respectively, exceeded the lowest maximum permissible limits set for molluscs of 1.5 µg/g d.w, prescribed by **FDA (2001)** and **WHO (1989)** and other organizations. Similar observations were recorded for iron (Fe) and zinc (Zn) (Table 8) which exceeded the lowest prescribed permissible limits by **WHO (1989)** and **FAO (1992)**. High levels of zinc cause pancreatitis, anemia, muscle pain and acute renal failure **(Pais and Benton Jones, 1997)**. The concentration of Cu and Ni were less than the lowest prescribed permissible limits (Table 8).

The concentrations of heavy metals differed between the studied species. **Szefer *et al.* (1999)** recorded significant interspecies variation in metals accumulation in the soft tissue of molluscs species collected from the Gulf of Aden, Yemen and this agree with the present study. The concentration of Cu, Fe and Ni were high in *R. decussatus* than *P. undulat*a and *V. pullastra* while Pb and Co recorded high values in *V. pullastra*. Temporal variations in heavy metals accumulation may be attributed to different factors such as food supply for the mollusc populations and/or runoff of particulate metals into the coastal waters and to various environmental (physicochemical conditions of water) and biological factors (physiological state of organism) **(Otchere *et al.*, 2003; Sokolowski *et al.*, 2004)**.

The concentration of studied metals for studied species in the present study were more or less comparable with those recorded in mollusca species by **El-Moselhy *et al.* (1999);** El-**Moselhy and Gabal (2004)**; **El-Moselhy and Yassien (2005)**; **Kesavan *et al.* (2013)**; **Sharaf and Shehata (2015)**. The variation in metals content in the different mollusca species may be attributed to the bioavailability of each species to uptake metals from the surrounding areas **(El-Moselhy*et al.*, 2016)**.

Current results revealed that the highest metal concentrations were Fe and Zn while the lowest concentrations were Cu and Ni in all studied species. This result may be due to the increasing of these metals into the lake. These findings are in agreement with previous observations recorded by **Ibrahim and El-Regal (2014); Sharaf and Shehata (2015); Abd El-Azim *et al.* (2018)** who studied the heavy metals concentrations in Lake Timsah, and found that Fe reached the maximum values in the soft tissues of the studied bivalve species.

On studying the relationship between size and heavy metal concentration, it was observed that the smallest *R. decussatus, P. undulat*a and *V. pullastra* contained the highest levels of heavy metals (Cu, Fe, Pb, Co, Ni and Zn). This is in agreement with **Cossa *et al.* (1980)**; **Joiris and Azokwu (1999)**. **Williamson (1980)** 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. **Usero *et al.* (1997)** noticed that independence between metal concentrations and size occurs when the uptake and excretion rates of the metals balance.

Balaji and Rao (2000) found negative correlation between concentration of Cu, Pb and Zn and size of *Mytilopsis sallei*. These results agreed with the present study except *V. pullastra* which had positive correlation between Cu and Fe concentration with its size. Also **Nik *et al.* (2014)** studied the relation between the concentration of heavy metals and the size of different species (*Donax cuneatus*, *Perna viridis*, *Anadara granosa* and *Cryptomya elliptica*) from Kuala Selangor, Malaysia. He found negative correlation between metal concentration (Fe, Pb and Zn) with clam size. This result agrees with the present study for all species except Fe in *V. pullastra*.**Yap *et al.* (2009)** suggested that there might be differences in physiology between young and older mussels. Since large and aged mussels tended to pump less water, through their bodies per unit of body weight, the uptake of metals was lower than that in smaller individuals. The surface area to volume ratios decreased with size, and this affected the relative contribution of the adsorbed metal content to the total body burden of heavy metals **(Swaileh and Adelung, 1994; Cossa *et al.*, 1997)**.

EL-Moselhy and Yassien (2005) studied the relationships between size of the two bivalve species and the concentration of heavy metals. They recorded that all metals have negative correlations with shell-length in studied species *(P. undulata* and *G. pectinatum*). These results agreed with the present study. These inverse relationships can be attributed to different reasons: 1) variation in the uptake and excretion rates of metals between small and large animals, 2) small animals have ability to eat more than the larger one which lead to accumulate pollutant in its body, 3) dilution of the pollutant in the tissues of the large animals. In general, it can be stated that, large individuals of the studied bivalve species in the present study are able to regulate the metals content in their tissues.

AbdElGhany (2017) studied the relationships between size of *Venerupis decussata* and the concentration of heavy metals (Cu, Fe, Pb, Co, Zn and Cd). He recorded that, all metals have negative correlations with shell size in study species. These results agreed with the present study. **Ibrahim and El-Regal (2014)** studied the relationships between shell length of two species (*Venerupis aurea* and *Thais carnifira*) and the concentration of heavy metals (Cu, Fe, Pb, Mn, Zn and Cr). They reported that there is negative correlation with *V. aurea* and positive correlation with *Thais carnifira.*

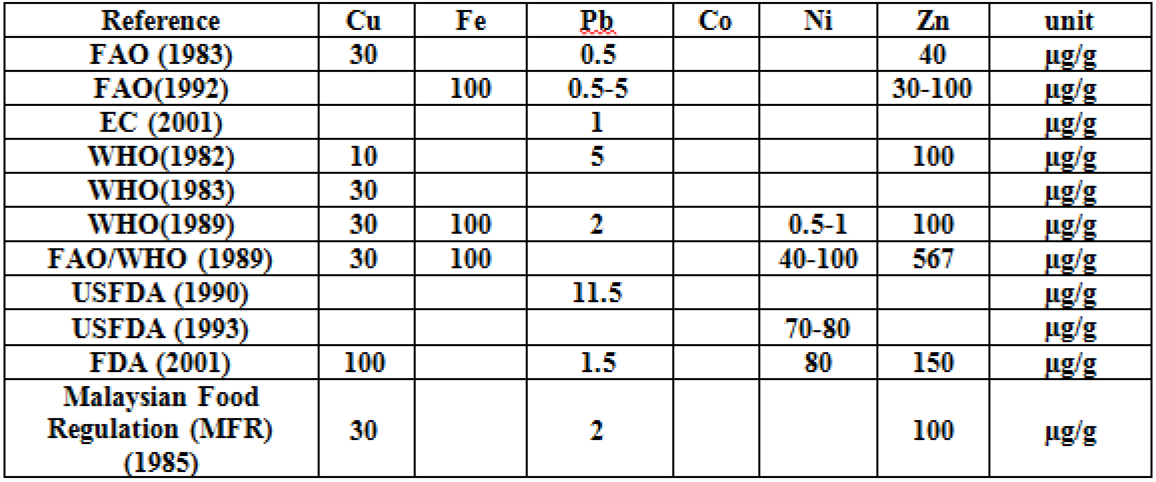
The accumulation and depuration kinetics is dependent on several factors, namely the type of contaminant, the sources of contamination (e.g., sediment, water, food), the conditions of exposure (i.e., controlled versus field), the bioavailability of the contaminant, etc **(Cardoso *et al.*, 2015)**. Unfortunately, little information on this topic is available in the literature, especially concerning the effect of different parameters on depuration rate of the commercial clams; however the present study is one of the few works that can surpass this knowledge gap.

Several trials make depuration by transplanting the clams or oysters in another clean field **(Saed *et al.*, 2004; Gabr and Gab-Alla, 2008)**. But these trials needed long periods for depuration that ranged from 50 days to 6 months. Other studies under took depuration in different experimental conditions **(EL- Shenawy, 2004; Saed *et al.*, 2004; El-Gamal, 2011)**. They found that it needs 2-32 days for complete depuration. These results referred that experimental depuration were faster for reducing the metal contents in bivalves. The present study suggests that depurating edible clams before their use for a period of three days is quite reasonable. This could be achieved in markets before being sold to avoid human toxicity with different pathogenic bacteria and heavy metals.

Previous studies reported higher and lower Cu, Fe, Pb, Co, Ni and Zn reductions in other bivalve species (*Paphia undulata, Ruditapes decussatus, Crassostrea gigas* and *Mytilus smarangdium*) subjected to depuration compared to the present study **(Gnassia-Barelli *et al.*, 1995; El- Shenawy, 2004; El-Gamal, 2011)**. These results clearly indicate that the pattern and efficiency of depuration rates of toxic and macro/trace elements are influenced by species, time of depuration, element, and initial elemental levels in bivalves and concentration of elements in the water of the depuration facility.

The obtained results from the present study suggest that small individuals of the studied species are faster in elimination of all heavy metals than the largest ones. This idea is similar with bioaccumulation of heavy metals with different clams size where the previous studies revealed that there are negative correlation between metal concentration and clam size **(Moselehy and Yassien, 2005; Ibrahim and Abu El-Regal, 2014).**

Table 8: The permissible limits for heavy metals set by different organizations.



Reference	Cu	Fe	Pb	Co	Ni	Zn	unit
FAO (1983)	30		0.5			40	µg/g
FAO(1992)		100	0.5-5			30-100	µg/g
EC (2001)			1				µg/g
WHO(1982)	10		5			100	µg/g
WHO(1983)	30						µg/g
WHO(1989)	30	100	2		0.5-1	100	µg/g
FAO/WHO (1989)	30	100			40-100	567	µg/g
USFDA (1990)			11.5				µg/g
USFDA (1993)					70-80		µg/g
FDA (2001)	100		1.5		80	150	µg/g
Malaysian Food Regulation (MFR) (1985)	30		2			100	µg/g

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