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Effect of temperature, feeding and starvation on depuration of heavy metals in some commercial bivalves of Timsah Lake, Suez Canal, Egypt

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ABSTRACT

The edible clams in the Timsah Lake are exposed to different industrial wastes which may be connected to the high accumulation of heavy metals in the studied species. Samples were collected from Timsah Lake in February 2019. The depuration experiments were commenced within 4 hours of the collection of shellfish. The lowest concentrations of Pb, Co, Ni and Zn after depuration were recorded at 15°C for *R. decussatus* while the lowest Pb, Co, Ni and Zn concentrations were recorded at 15°C, 25°C, 25°C and 20°C, respectively, for *P. undulata*. Significant differences were recorded in the reduction rate of heavy metals in the studied species with different temperatures except for Cu (P>0.05). The highest reduction rates of Cu, Fe, Pb, Co, Ni and Zn were recorded at 25°C, 25°C, 15°C, 15°C, 15°C and 15 °C, respectively, for *R. decussatus*. The highest depuration and reduction rates for all concentrations of heavy metals were determined at starvation trials in *R. decussatus* (except for Cu and Zn). The current study aimed to evaluate the effect of temperature, feeding and starvation on the reduction of heavy metals (Cu, Fe, Pb, Co, Ni and Zn) in some commercial bivalves; namely, *Ruditapes decussatus*, *Venerupis pullastra* and *Paphia undulata*.

INTRODUCTION

Venerid clams represent one of the most successful bivalves that have great commercial importance in the Suez Canal Lakes **(Fouda & Abou Zied, 1990; Mohammed *et al*., 1992; Mohammad & Yusuf, 2016)**. In Egypt, clams gain intensive demands in the local markets for their cheap price, great taste and flavor. Heavy metals were recorded in high concentrations in sediment, water and inhabiting biota as a result of industrial effluents discharged into the Timsah Lake **(Ibrahim & Abu El- Regal, 2014; Marwa & El-Hak, 2017; Sami, 2020; Dar *et al*., 2021)**.

Some factors affect the bioaccumulation rate of metals in bivalves; these factors may be biotic such as physiology status of species, sex, age and body weight or abiotic such as physical factors (temperature, salinity and pH) and chemical factors **(Fernandez-**

Tajes *et al.*, 2011). Therefore, there is a great interest in bivalve aquaculture in Egypt to afford substitute the source of free pollutants in bivalves and to meet the increasing demand for this sea food **(El-Wazzan & Radwan, 2013)**.

Depuration rates of clams were affected by many factors, including type of species, body size, activity of siphoning, shellfish size to water ratio flow rate and oxygenation **(Lee *et al*., 2010; Anacleto, 2014)**. Most depuration studies exposed shellfish to heavy metals in the laboratory and then transported them to clean water **(Wahi *et al*., 2009)** but few of them used naturally heavy metal contaminated clams and then followed their depuration in a clean field **(El-Shenawy, 2004; Sami *et al*., 2020)**. No recent information was found with respect to the impact of different parameters, such as temperature, feeding and starvation on depuration of these clams. Thus, the current study was conducted to evaluate the temperature effect, feeding and starvation on the elimination of some heavy metals in the commercial bivalves, including *Ruditapes decussatus*, *Venerupis pullastra* and *Paphia undulat*a, and prove the survival of bivalves during depuration.

MATERIALS AND METHODS

Sample collection

Clam samples were collected directly from fishermen using sieves in February 2019. In the laboratory, samples were washed with deionized water to get rid of any adhering contaminations, and then drained using filter paper. Shell lengths were measured by using a Vernier caliper with an accuracy of 0.01 mm. The soft part of bivalves was carefully detached with a plastic knife and was then enclosed in plastic bags and freezed at -4 ℃ until examination.

Depuration experiments

The depuration experiments were commenced within 4h of shellfish collection. The water used in all experiments was synthetic, with salinity of 25 ‰, and the water was 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) were measured in clams. The survival and mortality rates of bivalves were recorded daily during the study period.

Experiment 1: Effect of temperature on depuration of heavy metals in studied species

Twenty seven plastic tanks (3 L) (9 tanks each species) were used in this experiment. The temperatures used in this experiment were 15, 20 and 25 ℃. The experiments were carried out in three replicates for each tested temperature. Depuration process was studied for three days. Specimens were stocked (25 individuals per plastic tank) with different size classes from 25 – 30 mm for both species; *R. decussatus* and *P. undulata* and from 20 - 25 mm for *V. pullastra*.

Experiment 2: Effect of feeding and starvation on depuration of heavy metals in studied species.

Eighteen plastic tanks (3 L) (6 tanks each species) were used. The experiment was implemented at constant temperature (15 ± 1 ℃). The experiments were carried out in three replicates for each feeding and starvation with 3 days depuration time. Specimens were stocked (25 individuals per plastic tank) with different size classes (20 – 25 mm) for *R. decussatus* and *V. pullastra* and from 30 - 35 mm for *P. undulata*. Clams were fed the microalgae *Nanochloropsis* during the experiment.

Determination of heavy metals concentration

First, soft tissues of samples were dried in an oven at 70 ℃ until constant weight was reached. The concentrations of heavy metals (Cu, Fe, Pb, Co, Ni and Zn) were analyzed by using Atomic Absorption Spectrometry **(UNEP/FAO/IAEA, 1982)**. An amount of 10 ml of concentrated nitric acid was added to 0.5 g of dry tissue then heated at 90 ℃ to reach the complete digestion. The temperature was gradually increased to reach 135 ℃, and few drops of H_2O_2 were added for excess oxidation. Finally, samples were left for cooling, then diluted with distilled water and filtered by using 1.6 µm fiberglass filter paper (GF/A).

Metal depuration rate (µg/g day^{-1})

Metal depuration rate was calculated according to the equation of **Yap *et al*. (2003)** as follows:

$$\text{Metal depuration rate} = \frac{\text{Metal level before depuration} - \text{Metal level after depuration}}{\text{Days of depuration}}$$

Metal reduction rate (%) was calculated according to the following equation

$$\text{Metal reduction rate (\%)} = \frac{\text{Metal level before depuration} - \text{Metal level after depuration}}{\text{Metal level before depuration}} \times 100$$

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the significant differences between the concentrations of heavy metals in the three studied species as well as between the zero and the third day of depuration. If significant differences were present, Tukey's HSD was tested determining the differences between means at $p<0.05$ using SPSS program (Version 22).

RESULTS

Experiment 1: Effect of temperature on depuration of heavy metals in studied species

The concentrations of heavy metals before and after depuration with different temperatures for studied species are illustrated in Figs. (1- 3). The concentrations of all heavy metals for studied species after 3 days of depuration were significantly decreased compared to the initial concentration. There were significant differences in the concentration of heavy metals in studied species after depuration by changing the temperatures. The lowest concentration of Cu after depuration was recorded at 25 ℃, 15 ℃ and 20 ℃ for *R. decussatus*, *P. undulata* and *V. pullastra*, while the lowest Fe concentration was recorded at 25 ºc for all studied species. No significant difference was detected between the Cu concentration at temperatures 20 ℃ and 25 ℃ for *R. decussatus* and *P. undulate*. In addition, Fe showed no significant difference between temperatures of 15 ℃ and 20 ℃ for *R. decussatus*. The lowest concentrations of Pb, Co, Ni and Zn after depuration were recorded at 15 ℃ for *R. decussatus*; while, the lowest concentration of Pb, Co, Ni and Zn were recorded at 15 ℃, 25 ℃, 25 ℃ and 20 ℃, respectively, for *P. undulata.* The lowest concentrations of Co, Ni and Zn after depuration were recorded at 15 ℃ for *V. pullastra*, while Pb concentration was registered at 20 ℃.

The Reduction rate (%) and depuration rate (µg/g day^{-1}) of heavy metals with different temperatures for studied species are presented in Tables (1, 2). There were significant differences in the reduction rate of heavy metals in the studied species with different temperatures except for Cu (P>0.05). The highest reduction rates of Cu, Fe, Pb, Co, Ni and Zn were recorded at 25 ℃, 25 ℃,15 ℃,15 ℃,15 ℃ and 15 ℃, respectively, for *R. decussatus*. For *P. undulata*. Whereas, the highest reduction rates of Cu, Fe, Pb, Co, Ni and Zn were determined at 15 ℃, 25 ℃,15 ℃,25 ℃, 25 ℃ and 20 ℃, respectively. The reduction rates of Cu, Fe and Pb were high at 20 ℃, 25 ℃ and 20 ℃, respectively, in *V. pullastra*, while in Co, Ni and Zn the rates were high at 15 ℃ for the same species.

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

Metal	Depuration rate (µg/g dry weight day^{-1})								
	R. decussatus			*V. pullastra*			*P. undulata*		
	15 ℃	20 ℃	25 ℃	15 ℃	20 ℃	25 ℃	15 ℃	20 ℃	25 ℃
Cu	1	1.78	1.85	0.51	0.74	0.67	1.2	0.14	0.62
Fe	36.54	36.25	48.69	23.7	20.74	29.30	59.27	48.72	61.54
Pb	6.24	2.48	0.27	15.9	23.95	13.88	11.4	9.83	3.14
Co	6.48	4.66	1.95	3.71	3.53	2.90	2.56	3.65	3.95
Ni	7	2.8	2.45	6.77	6.24	5.18	6.35	8.63	9.23
Zn	15.82	11.61	6.33	14.09	10.29	6.96	17.47	32.73	3.53

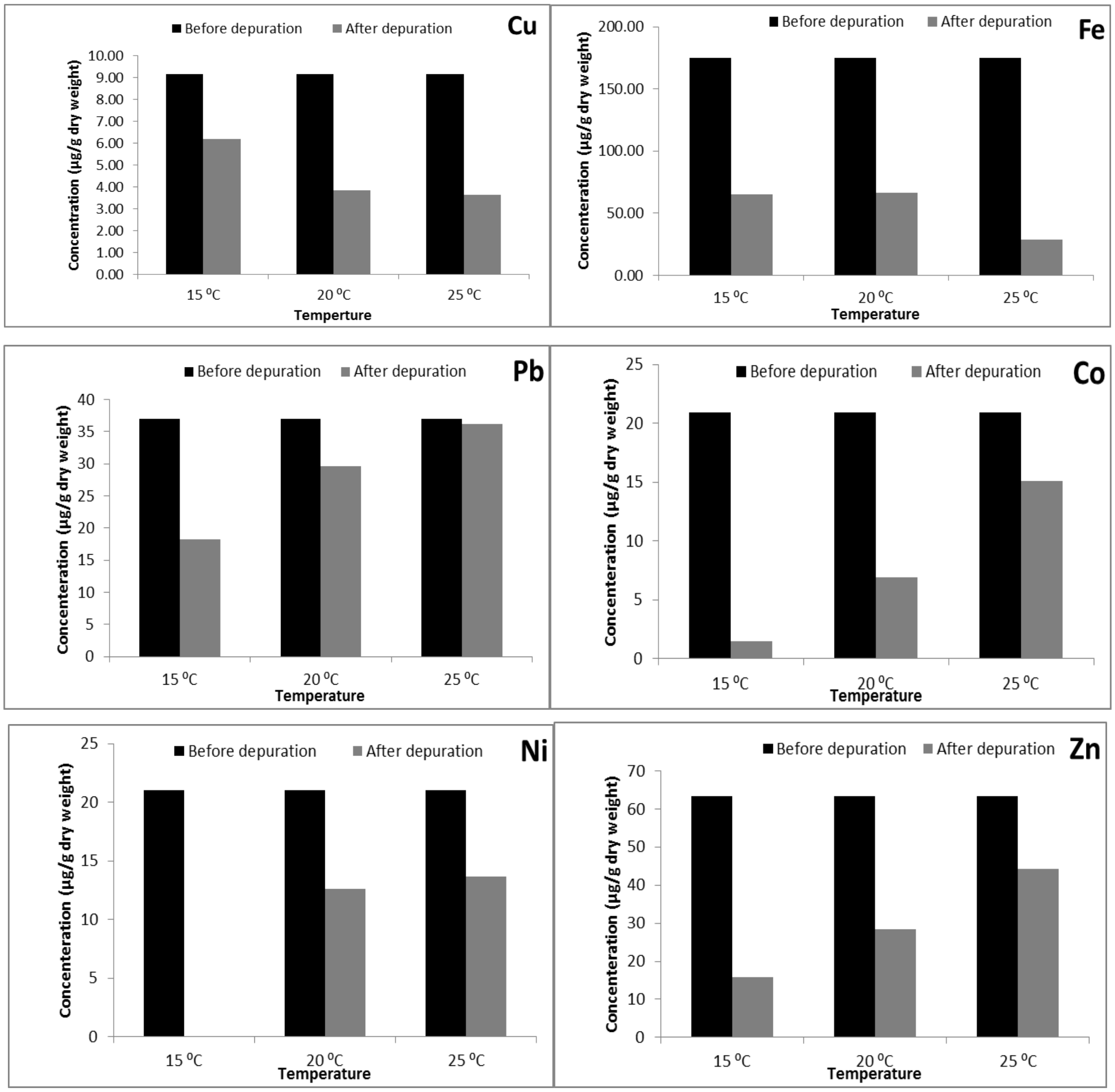

Fig. 1. Mean concentration (µg/g dry weight) of heavy metals in *R. decussatus* before and after 3 days – depuration

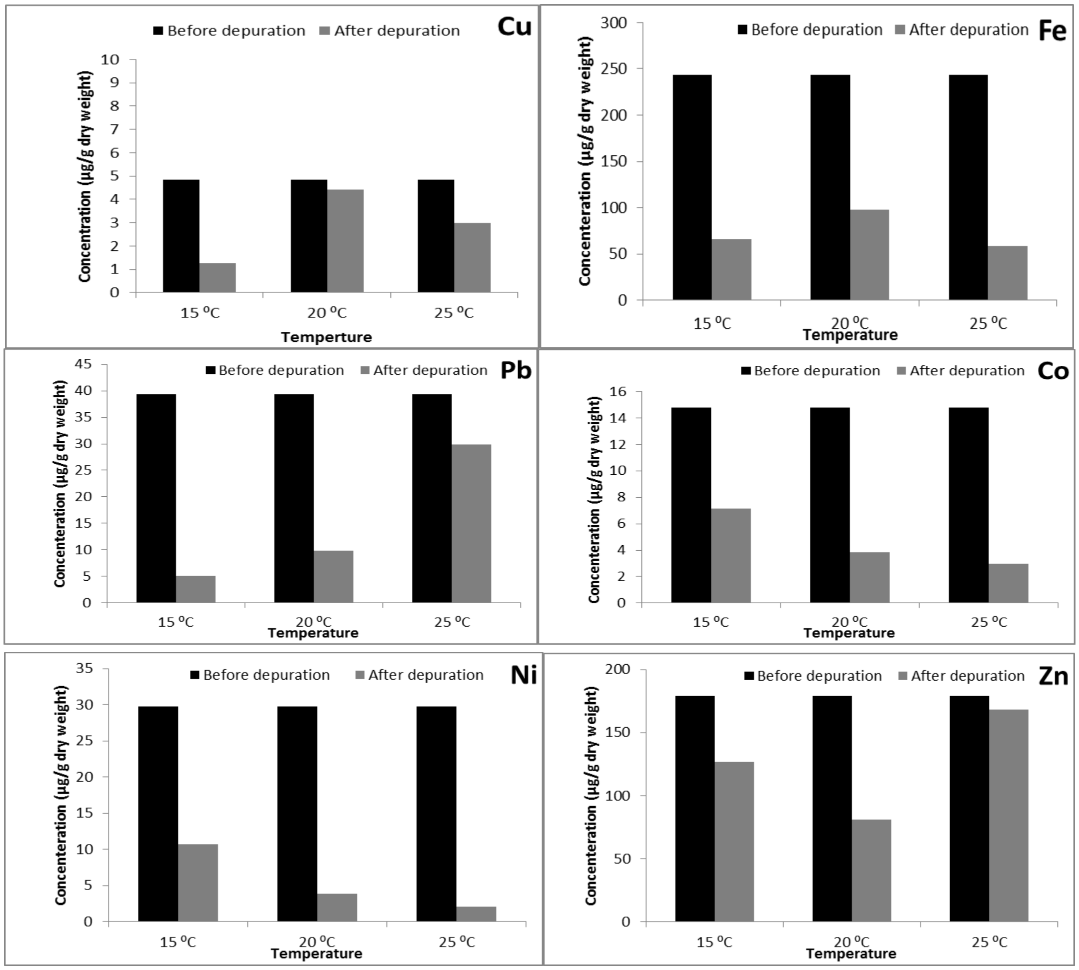

Fig. 2. Mean concentration (µg/g dry weight) of heavy metals in *P. undulata* before and after 3 days – depuration

Table 2. Reduction rate (%) of heavy metals in the soft tissues of studied species through different temperatures

Metal	Metal reduction rate (%)								
	R. decussatus			*V. pullastra*			*P. undulata*		
	15 °C	**20 °C**	**25 °C**	**15 °C**	**20 °C**	**25 °C**	**15 °C**	**20 °C**	**25 °C**
Cu	32.64	58.19	60.48	38.97	56.92	51.2	74.02	8.66	38.35
Fe	62.63	62.14	83.46	72.0	63.0	89.0	73.0	60.0	75.80
Pb	50.61	20.10	2.19	59.77	90.0	52.17	87.0	74.99	23.99
Co	92.97	66.86	27.98	80.17	76.42	62.80	51.92	74.0	80.01
Ni	100	39.98	34.94	83.99	77.41	64.29	64.01	87.0	93.01
Zn	75.01	55.03	29.99	66.95	48.91	33.09	29.28	54.85	5.91

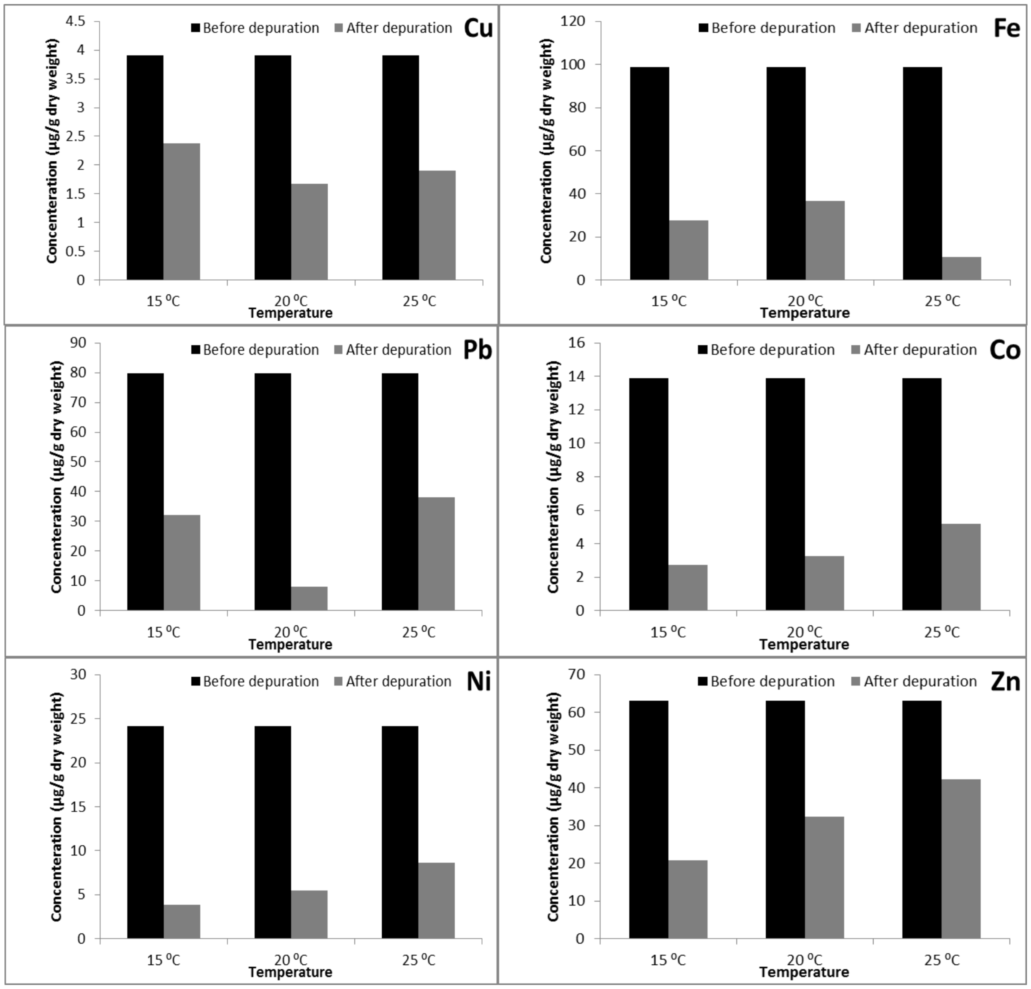

Fig. 3. Mean concentration (µg/g dry weight) of heavy metals in *V. pullastra* before and after 3 days – depuration

Experiment 2: Effect of feeding and starvation on depuration of heavy metals in studied species

Heavy metals concentrations before and after depuration with starvation and feeding trials for species under study are illustrated in Figs. (4- 6). The concentration of all heavy metals for the studied species after 3 days of depuration were significantly decreased compared to the initial concentration ($P<0.05$). Significant difference was noticed between the concentration of all heavy metals with starvation and feeding trials in all studied species. The highest depuration and reduction rates for all concentrations of heavy metals were recorded at starvation trials in *R. decussatus* (except Cu and Zn). The highest depuration and reduction rates for the concentrations of Cu, Fe, Pb and Zn were recorded at starvation trials for *P. undulate*. The lowest values for Co and Ni were assessed at starvation trials for *P. undulata* (Tables 3, 4). The highest depuration and reduction rates for all the concentrations of heavy metals were defined at feeding trials for *V. pullastra* (Tables 3, 4).

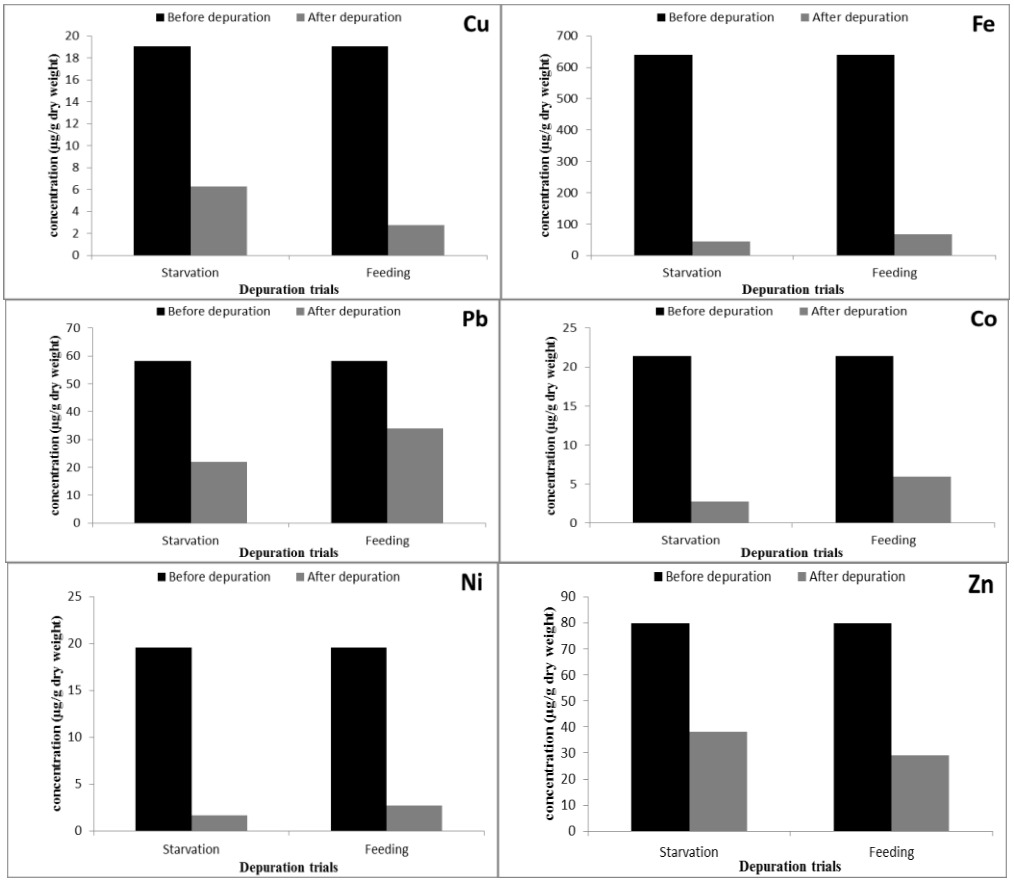

Fig. 4. Mean concentration (µg/g dry weight) of heavy metals of *R. decussatus* before and after 3 days – depuration with starvation and feeding

Table 3. Depuration rate (µg/g dry weight day^{-1}) of heavy metals in studied species through starvation and feeding trials

Metal	Depuration rate					
	R. decussatus		***V. pullastra***		***P. undulata***	
	Starvation	Feeding	Starvation	Feeding	Starvation	Feeding
Cu	**4.26**	**5.43**	**0.51**	**0.71**	**0.39**	**0.26**
Fe	**198.73**	**191.40**	**23.70**	**29.10**	**22.37**	**19.75**
Pb	**12.01**	**8.05**	**15.90**	**18.94**	**3.14**	**1.33**
Co	**6.21**	**5.14**	**3.71**	**4.10**	**1.32**	**2.11**
Ni	**5.97**	**5.64**	**6.77**	**7.79**	**1.31**	**1.89**
Zn	**13.83**	**16.89**	**14.09**	**16.31**	**2.64**	**1.25**

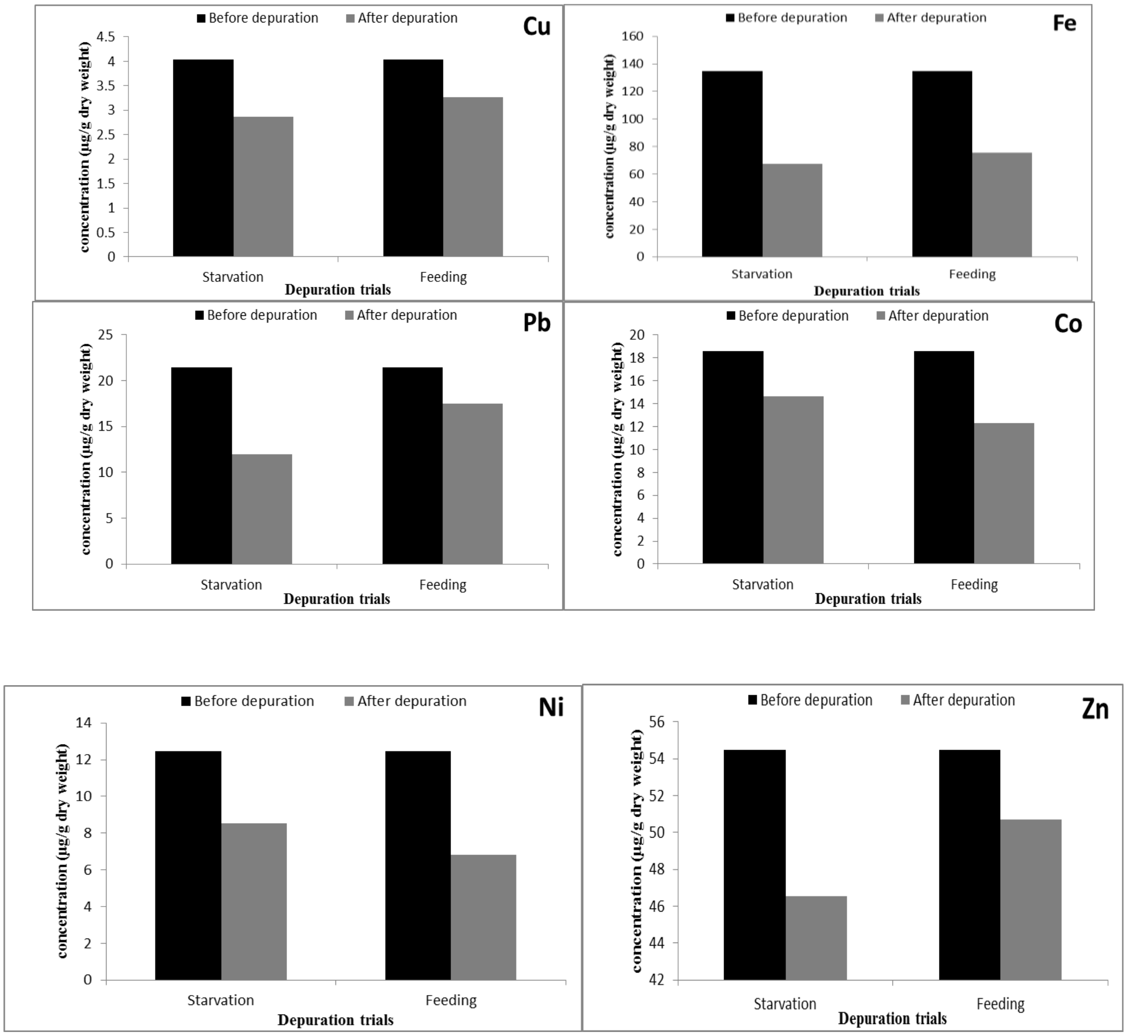

Fig. 5. Mean concentration (µg/g dry weight) of heavy metals of *P. undulata* before and after 3 days – depuration with starvation and feeding

Table 4. Reduction rate (%) of heavy metals in studied species through starvation and feeding trials

Metal	Metal reduction rate (%)					
	R. decussatus		*V. pullastra*		*P. undulata*	
	Starvation	Feeding	Starvation	Feeding	Starvation	Feeding
Cu	**67.12**	**85.61**	**38.97**	**54.87**	**29.21**	**19.31**
Fe	**93.07**	**89.64**	**72.00**	**88.39**	**49.84**	**44.00**
Pb	**62.03**	**41.58**	**59.77**	**71.20**	**43.98**	**18.58**
Co	**87.01**	**72.10**	**80.17**	**88.61**	**21.28**	**34.01**
Ni	**91.38**	**86.28**	**83.99**	**96.69**	**31.57**	**45.43**
Zn	**52.00**	**63.49**	**66.95**	**77.48**	**14.56**	**6.88**

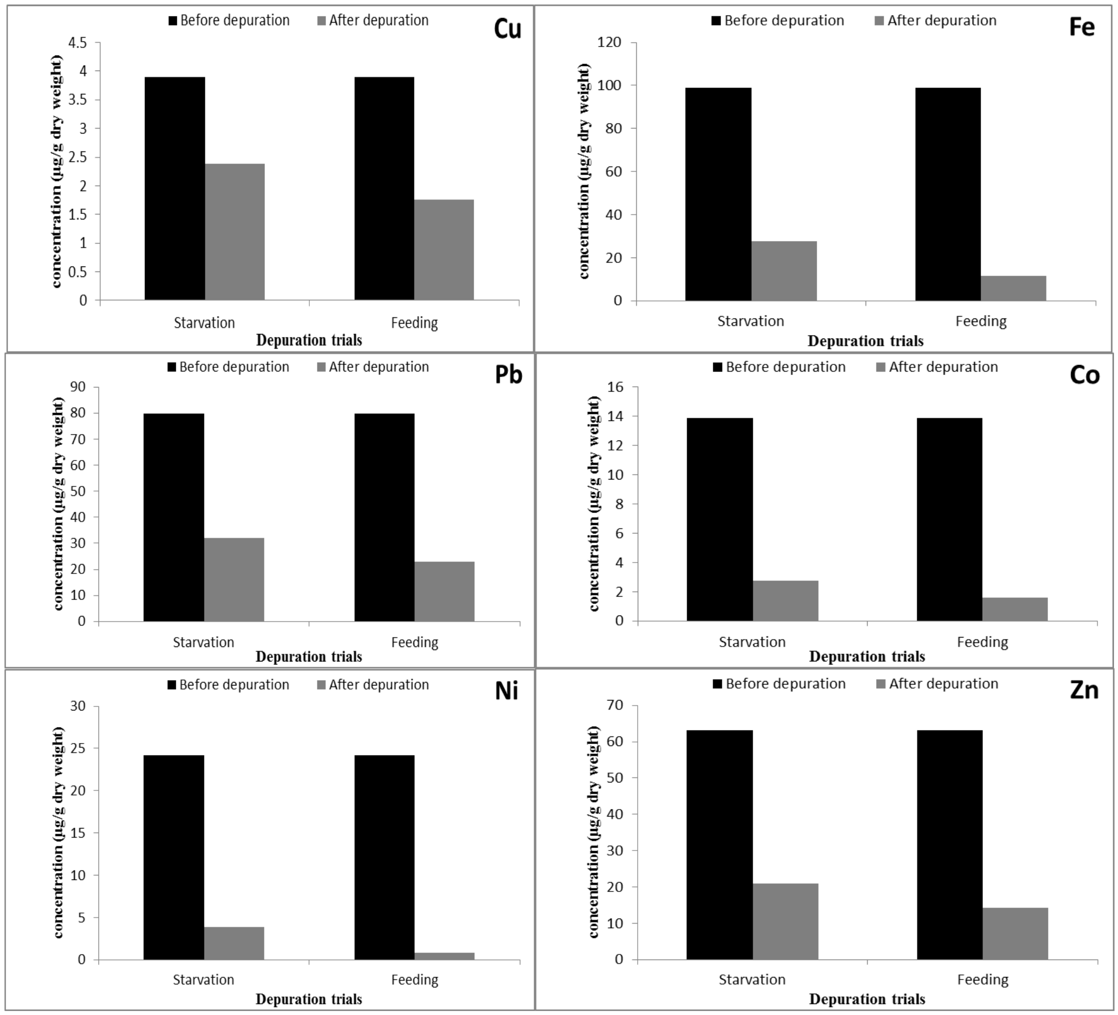

Fig. 6. Mean concentration (µg/g dry weight) of heavy metals of *V. pullastra* before and after 3 days – depuration with starvation and feeding

Survival rates (%)

A zero mortality rate occurred in the second experiment. In experiment 1, the survival rates ranged from 96 to 100% for *R. decussatus* and *V. pullastra*, while the survival rate of *P. undulata* ranged from 81.3 to 100 % (Fig. 7).

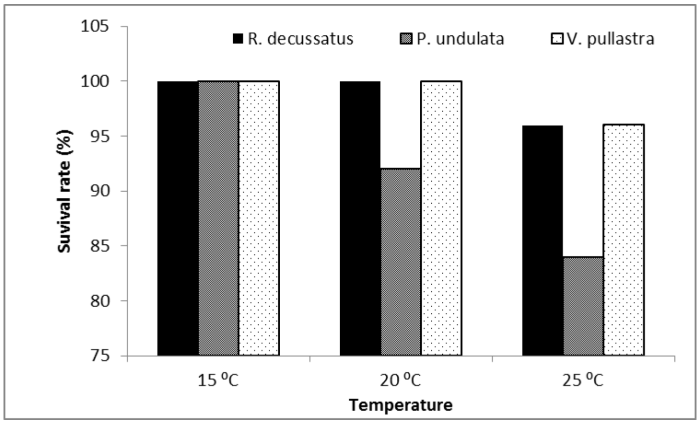

Fig. 7. Survival rate (%) for studied species in experiment 1 through the depuration period

DISCUSSION

The accumulation ratio of a metal in shellfish depends on many factors; some of them are originated from the surrounding environment such as temperature, pH, salinity, etc. **(Fernandez- Tajes *et al*., 2011; Phuong, 2014)**. While, some others are correlated to biological issues, such as sex, stage, age, maturation, etc., **(Mubiana *et al*, 2000)**. Bivalves have an important environmental role in water purification, filtering a considerable amount of water and sediment, which are correlated to their sizes while feeding on suspended particles > 2-3 µm **(Ponta *et al*., 2002)**. Nevertheless, marine animals greatly miss their activity in the heavy metal polluted environment, and this could clarify why depuration improve the metabolic state of bivalves **(El-Shenawy, 2004)**.

It was noticed that heavy metals accumulated by marine clams could be improved by increasing the temperature **(Phillips & Rainbow, 1993)**. On the other hand, it decreased in high salinity **(Eisler, 1981; Gibb *et al*., 1996)**. The rate of biological processes is greatly affected by increasing temperature, and this is not a condition to intensified metal bioaccumulation **(Phillips & Rainbow, 1993)**. Element accumulation is influenced by season and place **(Riget *et al*., 1996)** and clam size **(Sami *et al*., 2020)**. **Ray (1986)** reported that the bioaccumulation of cadmium increases with temperature, which in turn affects the host metabolism.

There is no much study on the effect of temperature on heavy metal depuration. The study of **Shuster and Pringle (1969)** indicated that metal depuration witnessed a more decrease at seawater temperatures of 4-12℃ than at 20℃. Mercury depletion in oysters increased when temperature was adjusted at 25 ± 2℃ and 79% of the remaining mercury vanishing after 80 days **(Cunningham & Tripp, 1975)**. Moreover, **Mandelli (1975)** stated that cold temperature situations were better for copper depuration.

Heart rate and water pumping of bivalves are regarded as indicators of general physiological fitness **(Cunningham & Tripp, 1975)**. Thus, more recovery of flesh components, including heavy metals would be predictable by increasing temperature and metabolism. This result was expected in the present study, where residence time of heavy metals in clams differed from one species to another and from metal to the other at the same temperature.

Lead is considered the second poisoning heavy metal. It aims special organs such as cardiovascular and reproductive systems, bones, brain, blood, kidneys and thyroid gland **(Homady *et al.*, 2002; Massadeh *et al*., 2004)**. Comparing the current data to the allowed parameters, it was observed that concentration of lead surpassed the lowest maximum limits for mollusks of 1.5 µg/g dry weight approved by **WHO (1989)**, **FDA (2001)** and other organizations. Concentrations of iron and zinc exceeded the lowest recommended limits of **WHO (1989)** and **FAO (1992)**. **Pais and Benton Jones (1997)** recorded that, the high levels of zinc concentration in human body cause anemia, pain of muscles, pancreatitis and severe renal failure. On the other hand, the concentrations of copper and nickel were less than the lowest approved limits.

The principal sources of lead recorded in the Egyptian Red Sea coasts are mainly from sewage and industrial inputs and water runoffs **(El-Sorogy *et al*., 2012)**. Lead absorption by sediment depends on grain size, content of organic materials and anthropogenic contamination **(Huang & Lin, 2003; Muniz *et al*., 2004)**. Remarkably, lead concentrations in the studied species were higher than those of **Gabr and Gab-Alla (2008)** who studied different heavy metal concentrations in *R. decussatus* and *V. pullastra* From the Timsah Lake, Egypt. The difference between the two studies are 13 years, thus it is expected that the concentrations increased due to anthropogenic contamination.

Previous studies of Cu, Fe, Pb, Co, Ni and Zn depuration in some clams (*Paphia undulata, Ruditapes decussatus, Crassostrea gigas* and *Mytilus smarangdium*), **(Han *et al*., 1993; Gnassia-Barelli *et al*., 1995; El- Shenawy, 2004; El-Gamal, 2011; Sami, 2020)** declared that the pattern and the efficiency of purification rates of toxic metals are affected by species, depuration time, metal, metal concentrations in shellfish, water of the depuration and clam size.

In experiment 1, the high reduction rates (%) of Cu, Fe, Pb, Co, Ni and Zn were recorded in *P. undulata, V. pullastra, V. pullastra, R. decussatus, R. decussatus* and *V. pullastra*, respectively, at 15, 25, 20, 15, 15 and 15℃, respectively. This may be attributed to the different physiological activities of the studied species and the different size clam, in addition that each species prefer specific temperature for eliminating the heavy metal from its body.

Overall, the present result revealed that the concentrations of all heavy metals were decreased in short depuration time (3days). The comparison of depuration process is difficult since the reduction or depuration rates differ among species. **Gabr and Gab-Alla (2008)** collected *R. decussatus* and *V. pullastra* from the Timsah Lake and transplanted them to farm (clean area) to reduce or eliminate the concentration of toxic elements. Their results showed that the concentrations of Cu, Fe, Pb, Co,Ni and Zn reduced with depuration rates of 0.07, 2.9, 0.008, 0, 0.03 and 0.44 µg/g day^{-1}, respectively, for *R. decussatus* and 0.05, 10.8, 0.01, 0.06, 0.05 and 0.1, respectively, for *V. pullastra*. These results were lower than those recorded in the present study. These differences in depuration rate could be due to different water parameters (temperature and salinity), clam size and concentration of heavy metals in water.

The highest depuration rate for all heavy metals in *V. pullastra* was observed in all feeding trials. These results may be related to the impact of feeding in triggering the animals to increase pumping rate and eliminate the metals from their body. Feeding did not affect depuration rate of all heavy metals except for Cu and Zn in *R. decussatus* and for Co and Ni in *P. undulat*a.

Bagenda *et al*. (2019) studied the effect of feeding oysters during prolonged depuration. They found that there is no significant effect on mortality. Usual mortality for oysters was 2.4% during feeding experiments; while, it was 4.1% in starvation trials. The rate of mortalities noticed during depuration was relatively low but not exclusive. This result agrees with that of the present study, where no evident mortality occurred through feeding and starvation trials.

Many studies perform depuration by transferring the shellfish in clean environment **(Saed *et al*., 2004; Gabr & Gab-Alla, 2008)**. Nevertheless, this technique needed long periods for depuration (50 days to 6 months). These results stated that experimental depuration were more rapidly for reducing the contents of toxic metals in bivalves. The present study recommends that depurating edible clams before their use for a period of three days is needed. This could be accomplished in markets before being sold to avoid human toxicity with different heavy metals.

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