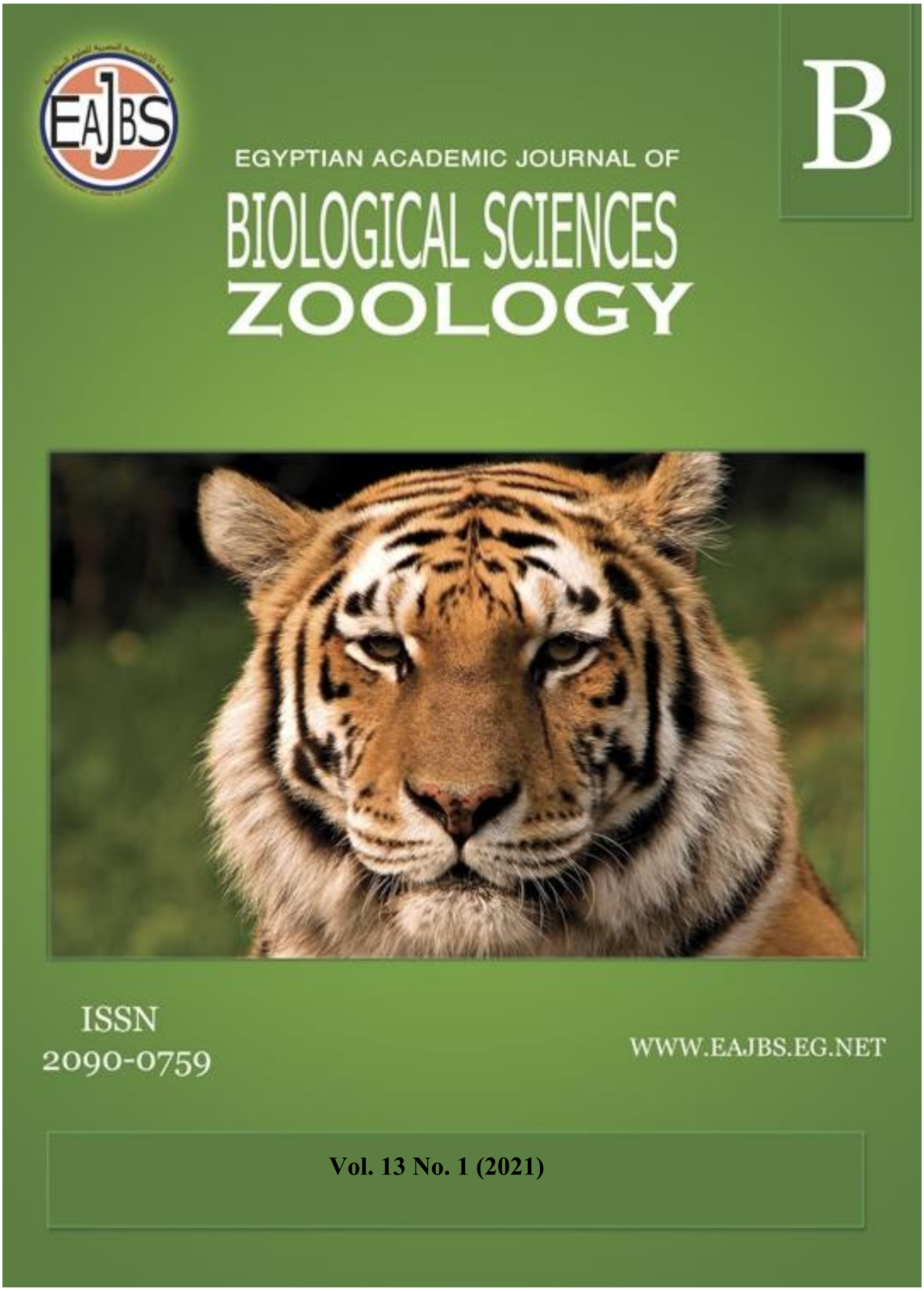

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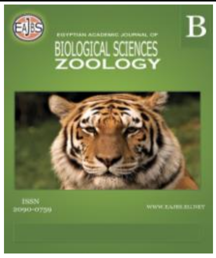

The Depuration Effect on Heavy Metals and Total Hydrocarbons Contamination Levels in *Donax trunculus* and Its Influence on The Expression of Oxidative Stress-Related Genes

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ABSTRACT

This study aims to investigate *Donax trunculus* (D. trunculus) as a biomarker for heavy metals and total hydrocarbon contamination. To achieve this goal, we investigated the effect of 3 day-depuration on the accumulation levels of heavy metals and total hydrocarbons as well as the transcriptional variations in expressions of oxidative stress-related genes of *Donax trunculus* collected from El-Gameel region, Port Said, Egypt. After 3 day-depuration, all the accumulated tested heavy metals levels showed a considerable decrease (levels after depuration divide by levels before depuration) in D. *trunculus* tissues by 23%, 20%,72%,98%, 89%, 66% for Pb, Cd, Cu, Fe, Mn and Zn respectively. Additionally, the concentrations of total hydrocarbons in clam's D. *trunculus* tissues were reduced to 95%. After 3 days of treatment, the results revealed that the Cat activity decreased to approximately 36% and expression of CYP gene had been up-regulated by about 38%, The Gst gene had been down-regulated by about 2-fold in D. *trunculus*. Additionally, Mt gene had been up-regulated to approximately 70% and SOD gene had been down-regulated to 50%. To conclude, accumulated heavy metals and total hydrocarbons measured before depuration in the soft parts of D. *trunculus* was higher than the standard worldwide acceptable limits leading to the hypothesis that D. *trunculus* in the investigated study area may not be safe for human consumption. Therefore, as a potential public health threat from the seafood diet, more research on the chronic toxic effects of heavy metals and total hydrocarbons in marine species are needed.

INTRODUCTION

Owing to their increased potency to move in and gather in the food chain, heavy metals and complete hydrocarbons are among the marine environment's most toxic pollutants. (Tam and Wong, 2000; Erdoğrul and Erbilir, 2007). Under specific environmental conditions in aquatic systems, heavy metals and hydrocarbons may accumulate to reach a toxic concentration resulting in ecological impairments (Jefferies and Firestone, 1984; Freedman, 1989).

The best strategy that has been established for bivalve risk controlling is to use their ability to eliminate pathogenic microorganisms and toxic substances when

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bivalves are kept in clean uninfected seawater tanks (Wong et al., 1997; Sobsey and Jaykus, 1999; El-Shenawy, 2004).

Some research used clams containing naturally high heavy metal concentrations and pursued their purification in a comparatively clean area (Okazaki and Panietz, 1981; El-Shenawy, 2004). Shellfish managed self-cleaning (depuration) is a common practice used to minimize microorganism loads. It is performed in managed seawater, which is permanently tracked by temperature, salinity, oxygenation and flow rate. In addition, the efficacy of the depuration cycle often depends on shellfish abundance and physiology, along with water and system features. (Schneider *et al.*, 2009).

The sensitivity of marine organisms to heavy metals can be indirectly measured through chemical analysis of seawater and sediment, but these results do not take into account the bioavailability of metals that rely on biological and abiotic factors (Khlifi and Hamza-Chaffai *et al.,* 1995). Researchers captured this natural phenomenon and found that low intertidal muscles altered their physiology related to the tide cycle very little, and mid-intertidal and high intertidal muscles decreased the gene expression involved in metabolic processes (Place *et al.,* 2012).

The depuration process aids bivalves to drive out and isolate contaminants from their gills and digestive tract over a duration of time avoiding over contamination. Although Arnold (1991) limited the role of this form of depuration in removing bacterial contamination, others recommended it and encouraged it to decrease heavy metal and hydrocarbons toxicity (Wilson, 1980; Hung *et al.*, 2001; El-Shenawy, 2004; Katayon *et al.,* 2004) and petroleum hydrocarbons (Rantamaki, 1997). Several criteria affect the degree of depuration such as system design, initial water quality, oxygenation and flow levels, salinity, temperature, shellfish-to-water ratios, faecal pollution removal and settlement, forms of contaminants in seawater and cleansing time (Lee and Younger, 2002; Manfra and Accorneo, 2005).

Bivalves are a prevalent and nutritious food source worldwide and have substantially increased demands on their intake. Since bivalves are filter feeders, they can possess toxins far higher than those in the surrounding aquatic ecosystem (Cosson, 2000; Fang *et al.,* 2003). These pollutants lead to human diseases as bivalves are often eaten raw or lightly cooked by many human communities. (Formiga-Cruz *et al.,* 2003). The best strategy that has been established for bivalve risk management is to use their ability to eliminate pathogenic microorganisms and toxic substances when bivalves are kept in clean uninfected seawater tanks (El-Shenawy, 2004).

Metal ions interfere with cell components such as DNA and nuclear proteins, resulting in DNA damage and conformation changes that lead to controlling of the cell cycle, carcinogenesis, or apoptosis (Kasprzak, 2002 Beyersmann *et al.,* 2008). Molecular studies have revealed that variations in gene expression can clarify specific cell responses in molluscs, as established by elevated metallothione (Mt) expression following exposure to toxic metals (Geffard *et al.*, 2005, Fasulo *et al.,* 2008). In a recent study, in response to oxygen depletion stress, differentially expressed genes (DEGs) and transcriptional changes were documented in several DEGs in marine mussels (Woo *et al.,* 2011). This research aims to estimate the efficacy of depuration on the removal of heavy metals and total hydrocarbons contamination level and their influence on the expression of oxidative stress-related genes.

MATERIALS AND METHODS

Sample Collection:

Samples were collected from a small portion of the lowlands west of Port Said City, known as the El-Gameel region, which stretches further west parallel to the Mediterranean Sea Deltaic Coast, 13 kilometers west of Port-Said City between latitude 31°10' - 31°20' N and longitude 32°00' - 32°20' E with about a 24 km coverage area. For the depuration experiment, samples were kept alive under laboratory conditions.

Depuration Process:

The depuration experiment was commenced after three hours of *Donax trunculus* collection Depuration process was studied for three and eight days. Four replicates of 25 clams (similar size) were placed in aquaria contained 5 litters of autoclaved artificial sea water (to be free of chemical contaminants under laboratory conditions (temperature 26 ± 2°C; salinity 29‰) and with continuous aeration. Water was changed twice daily.

Heavy Metal Determination:

The preparation of bivalve samples for trace metal analysis was taken place according to (UNEP/ FAO/ IAEA/ IOC 1984). Total P, K, Fe, Mn, Zn, and Cu in compost which were digested by aqua regia (hydrochloric acid and nitric acid) according to Cottenie *et al.* (1982) and determined by Inductively Coupled Plasma Spectrometry (ICP) (Ultima 2 JY Plasma), K was measured by flame photometer. For each run, three "blanks" were investigated using the same procedure.

Determination of Total Hydrocarbon:

Tissue samples were ground in a teflon mortar 2.0 g and each sample was extracted with two 25.0 ml of hexane. The samples were shaken on a shaker for 10 mins. A Whatman filter paper filtered the solution and the filtrate was diluted by putting 1ml of the extract into 50ml of hexane. This solution's absorbance was read at 430 nm using n-hexane as blank with Jenway 6405 UV / Vis spectrophotometer.

Total RNA Extraction and First Strand cDNA Synthesis:

The total RNA was obtained from *Donax trunculus* organs sampled at the point of collection. ($n = 5$). Tissue homogenate and RNA isolation were done using Qiazol reagent (Qiagen, USA) following the manufacturer's manuals. The RNA reliability was assessed on 1 % (*w*/*v*) agarose gel and the concentration and the purity confirmed by Nanodrop spectrophotometer (Thermo Scientific). Complementary DNA first-strand (cDNA) was created from 1 µg total RNA using the QuantiTect reverse transcription kit (Qiagen, USA) according to annuals of manufacturers, after elimination of any genomic DNA contamination as formerly described by Giannetto *et al.* (2013).

Determination of Transcriptional Response by qPCR:

Expression levels of the designated gene (housekeeping gene: β-actin, Catalase: cat, Cytochrome P450: CYP, Glutathione S-transferase: GST, Metallothionein: Mt, Superoxide dismutase: SOD) were quantified by real-time PCR using the Rotor-Gene Q 5plex Hrm thermocycler (Qiagen, USA) with SYBR Green chemistry (Qiagen, USA). The primers used for the qPCR are tabulated in Table 1. The relative gene expression of proposed genes was evaluated using the normalization factor from the housekeeping gene, β-actin gene (Giannetto *et al.,* 2015). Real-time PCR reactions were taken place in triplicate using diluted cDNA and controls (Lazado e*t al.* 2014). Q-PCR run conditions were as follows: 15 min at 95 °C, followed by two-step cycling of 5 s at 95 °C, and combined annealing/extension of 10 s at 60 °C followed by 15s at 72 °C for 40 cycles. PCR efficacy (*E*) and the specificity of the reaction were assessed as detailed in Giannetto *et al.* (2017).

Table 1: Primer pair sequences, amplicon size and GenBank accession number for quantitative real-time PCR analyzed genes. *Donax trunculus.*

Gene	Primer sequences	GenBank accession #	Amplicon size (bp)
β-actin	For '5'-AAGGCCAACCGGGAGAAGATG-3 Rev 5'-GGTCAGCAATGCCAGGGAAC-3'		
Catalase (cat)	For 5′-TGCTCTGGGATTTCATTAG3′ Rev 5′-CAGCACTCAGACATTTTATAC3′	AY743716	212
Cytochrome P450 (cyp4y1)	For 5′-GAGGCTTCATTCACCAGTTC3′ Rev 5′-GAGTAAATGCAAAAGAGTCC-3′	AF072855	212
Glutathione S-transferase (GST)	For 5′-AGAAAATTGGGTAGAAAACTGG-3′ Rev 5′-CATTCTAACGTAAGCCCCTCTG3′	AF527010	194
Metallothionein (Mt)	For 5′-TACCCAGATACCACCCATACT-3′ Rev 5′-GAACATCCACAGCCACTTG3′	AJ005456	188
Superoxide dismutase (SOD)	For 5′-AACAGTCGCTTTCAGTCAAC3′ Rev 5′-TACATTTCCCAGATCACCAAC3′	FM177867	214

RESULTS

Effect of Depuration on The Accumulation Level of Heavy Metals in The Soft Tissue of *D. trunculus:*

After 3 days of depuration, the content of toxic elements determined in Donax trunculus tissue is shown in Table 2. It was obvious that there is a significant reduction between all element concentrations before and after depuration except for Fe slight differences were detected. The initial concentration of Mn was the highest value with a mean of 556.03 µg/g reduced after depuration to 89% (495 µg/g) compared to its initial value, while the initial concentration of Cd was the lowest (0.1 µg/g) reduced to less than 20% (0.02 µg/g) of its initial value. Also, Pb, Zinc and Cu reduced to 63, 65% and 73%, respectively compared to their initial values. The lowest significant reduction value recorded in Fe initiated with 203.48 to 199.9 µg/g. As it is shown in Table 3, the mean concentration of Pb, Cd, Mn and Zn recorded a significant decrease after the depuration period comparing to their values recorded before the depuration period (Table 2).

Table 2: Mean concentrations (µg/g) of heavy metals in tissue of D. *trunculus* before and after 3 days-depuration.

	Before depuration	After depuration
Total hydrocarbons	81.70±0.83	78.20±0.88*

*, statistically significant comparing to before depuration values, $P < 0.05$

Effect of Depuration on The Accumulation Level of The Total Hydrocarbons in Soft Tissue of *D. trunculus:*

A significant reduction was recorded after three days of the depuration process as shown in Table 3. The total concentration of total hydrocarbons accumulated in tissue before depuration was (81.7 µg/g) which was reduced to 78.2 µg/g after the depuration process. The concentrations of total hydrocarbons in D. *trunculus* tissues were reduced to 95% compared to their initial concentrations. As it is shown in Table 3, the mean concentration of total hydrocarbons recorded a significant decrease after the depuration period comparing to their values recorded before the depuration period (Table 3).

Table 3: Mean concentrations (µg/g) of total hydrocarbons in soft parts of *D. trunculus* before and after 3 days-depuration

	Before depuration	**After depuration**
Pb	6.63±0.24	4.20±0.15*
Cd	0.10±0.04	0.02±0.01*
Cu	2.63±0.03	1.90±0.16
Fe	203.48±1.21	199.60±1.83
Mn	556.03±3.51	495.00±1.51*
Zn	303.38±1.06	200.20±1.80*

*, statistically significant comparing to before depuration values, $P < 0.05$

Transcriptional Changes of RNA Expression of Oxidative Stress-Related Genes of Exposed *D. trunculus* Before and After Depuration*:*

To determine the efficacy of the accumulation of heavy metals and hydrocarbons on *D. trunculus* physiology and biochemistry, *D. trunculus* were collected from the polluted area along El-Gamil coast and undergo depuration in Seawater for 3 days and the transcriptional changes of RNA levels of oxidative stress-related genes (Cat, Cyp, Gst, Mt and Sod) were recorded before and after depuration.

The fold change in transcriptional expression of RNA of oxidative stress-related genes tested in this study had varied in different genes after exposure of *D. trunculus* to depuration protocol. There were no-significant transcriptional changes of RNA expression of examined genes related to oxidative stress examined in this study were monitored following exposure to pollutants as heavy metals and hydrocarbons.

Generally, the transcription of Cat and Gst gene expression was slightly down-regulated, and the transcription of Gst and Sod genes displayed similar expression approaches, with to some extent down-regulation by 2-fold, after depuration comparing to their expression before depuration, while the transcription of Cyp, Mt was somewhat up-regulated to 38% and 70%, respectively comparing to their expression before depuration.

Transcriptional Changes *of RNA* Expression of Catalase (cat) Gene*:*

As it clearly demonstrated in Figure 1 that the transcriptional changes of RNA expression of CAT gene significantly down-regulated to approximately 36% in D. trunculus after 3 days of depuration comparing to its expression before depuration. Transcriptional value of RNA expression of Cat gene of exposed D. trunculus was significantly decreased after depuration period comparing to its value before depuration period (Fig. 1).

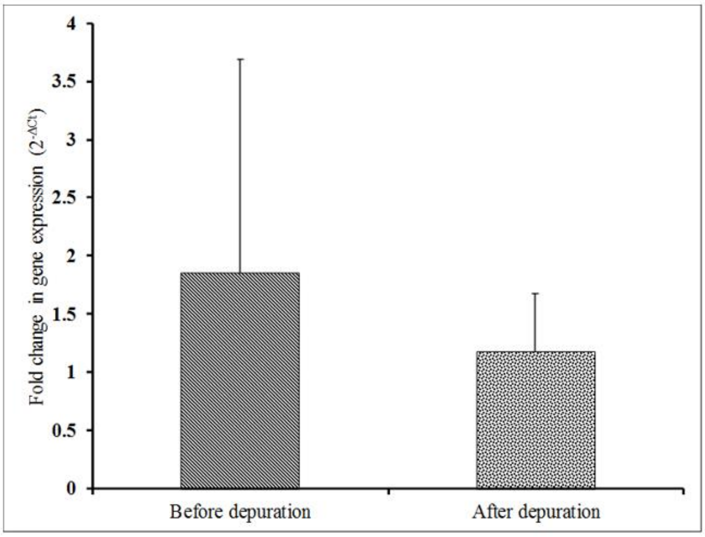

Fig.1: Transcriptional changes of RNA expression of Cat gene of exposed D. trunculus before and after depuration period.

Transcriptional changes of RNA expression of Cytochrome P450 (Cyp) gene.It is obviously clear from Fig. 2 that the transcriptional changes of RNA expression of Cyp gene up-regulated by about 38% in D. trunculus tissue after 3 days of depuration comparing to its expression before depuration. Transcriptional value of RNA expression of Cyp gene of exposed *D. trunculus* was significantly increased after depuration period comparing to its value before depuration period (Fig. 2).

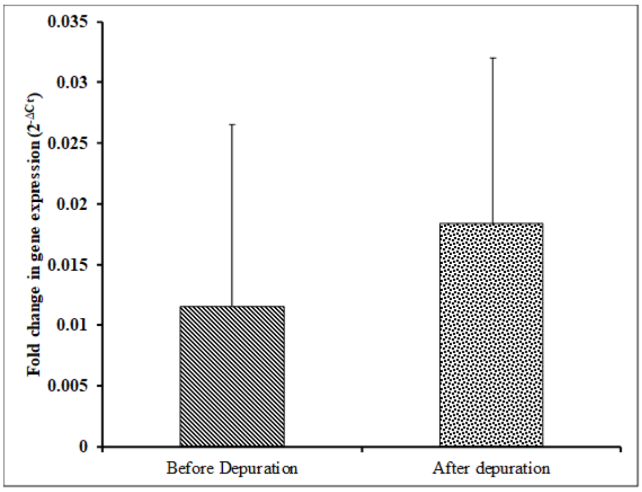

Fig. 2: Transcriptional changes of RNA expression of Cyp gene of exposed D. trunculus before and after depuration period.

Transcriptional Changes of RNA Expression of S-Transferase Glutathione (Gst) Gene*:*

The data in Figure 3 revealed that the transcriptional changes of RNA expression of Gst gene down-regulated 2-fold in *D. trunculus* tissue after 3 days of depuration comparing to its expression before depuration. Transcriptional value of RNA expression of Gst gene of exposed *D. trunculus* was significantly decreased after depuration period comparing to its value before depuration period (Fig. 3).

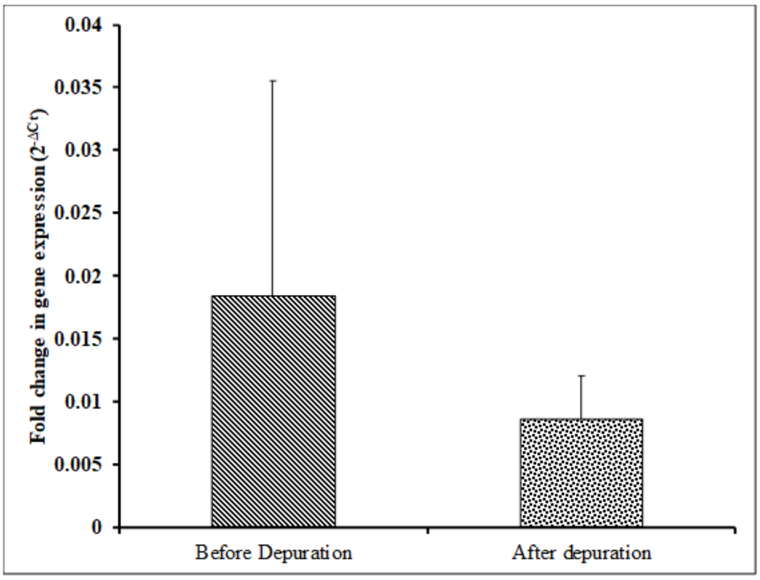

Fig. 3: Transcriptional changes of RNA expression of Gst gene of exposed D. trunculus before and after depuration period

Transcriptional Changes of RNA Expression of Metallothionein (Mt) gene:

The results in Fig. 4 postulated that the transcriptional changes of RNA expression of Mt gene up-regulated to approximately 70% in *D. trunculus* tissue after 3 days of depuration comparing to its expression before depuration. Transcriptional value of RNA expression of Mt gene of exposed *D. trunculus* was significantly increased after depuration period comparing to its value before depuration period (Fig. 4).

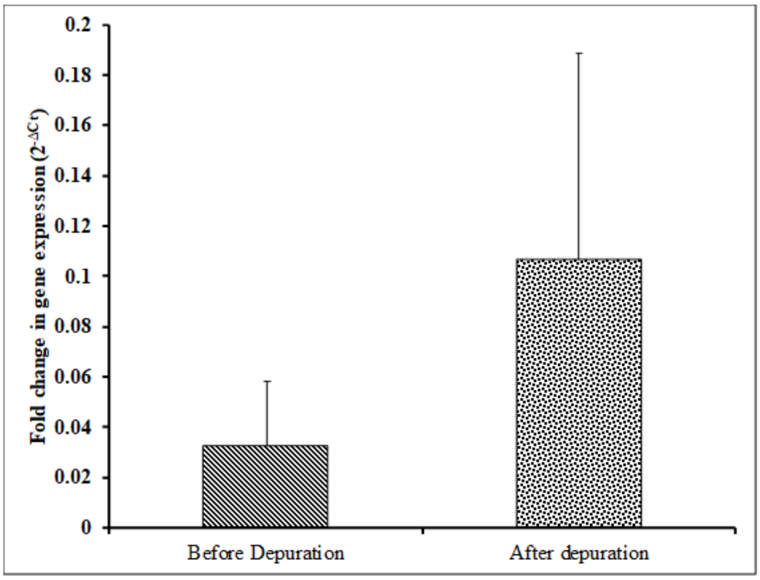

Fig. 4: Transcriptional changes of RNA expression of Mt gene of exposed *D. trunculus* before and after depuration period.

Transcriptional Changes of RNA Expression of Superoxide Dismutase (SOD) Gene*:*

The results in Fig. 5 postulated that the transcriptional changes of RNA expression of Sod gene up-regulated to approximately 70% in *D. trunculus* tissue after 3 days of depuration comparing to its expression before depuration. Transcriptional value of RNA expression of Sod gene of exposed *D. trunculus* was significantly decreased after depuration period comparing to its value before depuration period (Fig. 5).

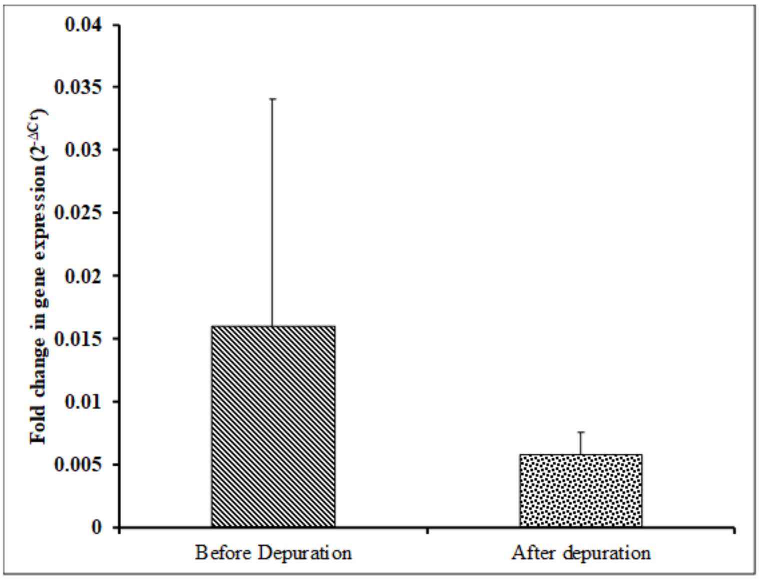

Fig. 5: Transcriptional changes of RNA expression of Sod gene of exposed D. trunculus before and after depuration period

DISCUSSION

Upon reaching the *D. trunculus* tissues, several factors control the accumulation of contaminants, e.g., their development in regard to primary food productivity, the trophic capacity of the environment, or lipid content. (Thuy *et al*., 2018). Lovejoy (1999) and Anandraj *et al.*, (2002) stated that molluscs have a depuration role in their body to decrease heavy metal toxicity, declining the efficiency of molluscs as biomonitoring organisms. Black *et al.*, (1997) formerly stated this. They also elucidated that clams could have vital enzymes or pathways for the progressions of detoxification or repair. Farid *et al.*, (2012) revealed that the bivalve can discharge enormously large quantities of petroleum hydrocarbons into the atmosphere via its binding mechanism for the mucus oil that it may be connected to different enzyme or defense mechanisms within each living organism to provoke and resolve the risks of pollution (El-Shoubaky and Mohammad, 2016).

In this study the initial concentration of Mn was the highest value with a mean (556.03 µg/g) decreased after three days of depuration (11% was depurated of its initial value) compared to its initial value. Our result was much less than El-Gamal (2011) who proposed that manganese was removed more rapidly after 24 h depuration, (83% of the initial concentration was depurated) than the other elements despite its high concentration in the sediment. Zn displayed a significant decrease to 65 % from its initial value. This shows that Zn has been controlled in *Donax trunculus*. This observation was reliable with that of Yap *et al*., (2003), which stated that *Perna viridis* also controls Zn after exposure. This comes with the fact postulated by Viarengo *et al.*, (1983), Zn is important for metabolism, but it could also be regulated in the bivalve organism (Phillips 1985). *D. trunculus* capacity to regulate Zn has limited its effectiveness to be used as a bio-monitor for Zn. This result agreed with Rashid *et al.*, (2009) who also resulted that *M. meretrix* would not be an effective bio-monitoring organism for Zn.

Our results revealed that Pb and Cu reduced to 63% and 73%, respectively compared to their initial values. They showed a high reduction ratio this may be because of their weak binding within the *D.trunculus* tissues. The lowest significant reduction value recorded in this study was Fe which initiated with (203.48 and become 199.9 µg/g) after three days of depuration. This result in line with Ruddell (1971) who clarified that metals that can be removed may be bound in amoebocytes and not fixed within the molluscan tissues. Roesijadi (1980) also related the high affinities of metals to bind with metallothionein to their capacity to fix themselves within the different tissues.

The concentrations of total hydrocarbons in clam's *Donax trunculus* tissues were decreased to 95% compared to their initial concentrations after three days of depuration under laboratory conditions. This decrease of the total hydrocarbon in tissue was less than that of El-Gamal (2011) who found that the duration of Paphia undulates accumulated with TPHs, it had a slow beginning at the start of the experiment (10% depuration after 24 h) followed by a faster release after 72 h (29%) to be 72% compared to their initial concentration. This comes with the fact that both the site of accumulation and the rate of accumulation of a particular hydrocarbon have an effect on the rate of depuration. Heavier species are accumulated more slowly, and the rate of accumulation is probably related to the molecular weight, configuration and type of components (Mason, 1988). Mason also found that there are further factors that will also affect the depuration rate, the solubility of components in seawater and in the tissue, lipid will affect the rate at which components are taken up and released. Heavy aromatics (e.g., benzopyrene) are relatively insoluble in both seawater and organic solvents, and therefore it would be predictable that these compounds would be comparatively insoluble in tissue lipids. Such a theory may account for the quicker loss of the heavier aromatics during depuration, whereas 2-4 ring aromatics, which are most soluble, are less rapidly lost. Metal accumulation is created by feeding; integrating the metal's bioavailable forms (Rainbow *et al.*, 1990). The presence of the lesions of the digestive gland shows prolonged contact through the oral route with the toxins and the failure to remove them.

The transcription levels of oxidative stress-related genes: Cat, cyp4y1, Mt, Sod and Gst of *D. trunculus* were quantitatively compared between specimens exposed to heavy metals and hydrocarbons and those that undergo depuration treatment. The genes involved in the protection of stress can be used as an effective biomarker of physiological changes in species arising from both endogenous and exogenous stressors. These gene expression levels may serve as a vital ' early warning system ' for assessing the health of the environment and species.

Our results revealed that the transcriptional level of the Sod, Cat and Gst RNA expression was significantly down-regulated upon exposure to heavy metals and hydrocarbons, while the transcriptional level o of Cyp and Mt RNA expression was up-regulated during exposure to heavy metals and hydrocarbons. Cat is the main enzyme and primary antioxidant defense involved in detoxification of H_2O_2 (catalyzing the conversion of H_2O_2 to water and oxygen), whereas Gst catalyzes a number of endogenous compound detoxification reactions, including peroxidized lipids. (Das and Bishayi 2010; Turkanoglu *et al*., 2010). It may be possible to interpret the disparity between the levels of CAT activity and cat messenger RNA (mRNA) by evaluating variations in protein levels using a CAT protein-specific antibody. Animals with decreased Cat activity may have severe hypoxia accompanied by reoxygenation (Welker *et al.*, 2012).

Heavy metals and hydrocarbons pollution-induced changes in the Sod and Gst transcription activity after depuration time in *D. trunculus* This showed that the decrease in CAT activity induced by pollutants produced a redox imbalance, which was necessary to trigger more oxidative stress that resulted in increased GST and LPO activity in moulds. (Fu *et al*., 2008).

The genes examined in the current research point are playing crucial approaches in detoxification and are vital factors in determining the sensitivity of *D. trunculus* tissues to various toxic chemicals, including environmental pollutants and products of oxidative stress. The data proposed that the variations in gene expression do not always correspond with enzyme activities. This can be clarified by biological responses such as variations in gene expression that arise in the initial stage following exposure to environmental stress and their consequent signals such as protein expression or changes in behavior depending on the strength of the stimuli.

Conclusion

In conclusion, it was clear that the contamination levels of heavy metals and total hydrocarbons in the tissue of *D. trunculus* tissues were markedly decreased. The content of heavy metal and total hydrocarbons measured before depuration in the soft parts of *D. trunculus* was higher compared to the standard worldwide acceptable limits leading to the hypothesis that *D. trunculus* in the investigated study area may not be safe for human consumption. Additionally, *D. trunculus* may be a good bio-indicator organism for heavy metals and total hydrocarbon contaminations. Therefore, further studies on chronic toxic effects of heavy metals and total hydrocarbons in marine organisms, especially edible bivalves, are needed as a possible public health threat from the seafood diet.

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ARABIC SUMMARY

تأثير التنقية على التلوث بالمعادن الثقيلة والهيدروكربونات الكلية في أم الخلول (*Donax trunculus*) وتأثيره على تعبيرات الجينات المرتبطة بالإجهاد التأكسدي

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هدفت الدراسة الحالية الي التحقق من دور حيوان ام الخلول والذي ينتمي الي الرخويات ثنائية المصراع كمؤشر حيوي لقياس مستوي التلوث البحري بالمعادن الثقيلة والهيدروكربونات الكلية؛ وعلية تم قياس تأثير عملية التنقية لمدة ثلاثة أيام متواصلة على تراكم مستوي المعادن الثقيلة والهيدوكربونات في انسجة حيوان ام الخلول بالاضافة الي دراسة معدل التغير الجيني لبعض الجينات المرتبطة بالاجهاد التاكسدي) الكاتليز و السيتوكروم وترانسفيريز جلوتاثيون و ميتالوثايون وسوبراوكسيد ديسماتيز) . تم تجميع العينات من منطقة الجميل بمحافظة بورسعيد وتوزيعها على ثلاثة احواض تحتوي على ثلاث لترات من الماء البحري المنقي والمحضر معمليا (درجة حرارة ٢٦±٢ ودرجة ملوحة ٢٩)ومع تهوية مستمرة وتغير الماء مرتين يوميا. أظهرت النتائج للعينات بعد عملية التنقية انخفاضا ملحوظا في مستوي المعادن الثقيلة المتراكمة في انسجة الحيوان مقارنا بنسبتها في بداية التجربة الي ٢٠%و٢٣%و٦٦% ٧٢و %٨٩%و٩٨% لكل من عنصر الكادميوم والرصاص والزنك والنحاس والمنجنيز والحديد على الترتيب.
كما ساهمت عملية التنقية في انخفاض ملحوظ لتركيزات لهيدروكربونات المتراكمة في انسجة الحيوان اقل بنسبة 5% من تركيزها في بداية التجربة. كما اثرت عملية التنقية على النشاط الجيني للكاتليز الي ٣٦% والسيتوكروم جين الي ٣٨% كما أظهر ترانسفيريز جلوتاثيون جين انخفاض بمقدار ضعفين مقارنا ببداية التجربة كما شهد النشاط الجيني لكل من ميتالوثايون جين وسوبراوكسيد ديسماتيزجين انخفاضا الي ٧٠%و٥٠% على الترتيب. ختاما؛ وفقا للنتائج المستخلصة من هذة الدراسة فان تركيز المعادن الثقيلة والهيدروكربون المتراكمة في انسجة أم الخلول والمجمع من منطقة الجميل بمحافظة بورسعيد. تعتبر اعلي من المعدلات العالمية المناسبة للاستهلاك الادمي. كما ابرزت دور أم الخلول كمؤشر بيولوجي لقياس معدلات التلوث البيئي.